Jurnal Sains Kesihatan Malaysia **9** (1) 2011: 29-34

Kertas Asli/*Original Article*

Changes in Nitrate and Nitrite Levels of Blanched *Amaranthus* During Refrigeration Storage

(Perubahan Kandungan Nitrat dan Nitrit dalam *Amaranthus* yang Dicelur Semasa Penyimpanan Sejuk)

CHEW SC, K. NAGENDRA PRASAD YANG BAO & AMIN ISMAIL

ABSTRACT

Changes in nitrate and nitrite contents (leaves and stem) of Amaranthus gangeticus (AG) and Amaranthus paniculatus (AP), resulting from blanching, storage time (0-4 days), storage temperature (0 and 4º C), and reheating were analysed. Results showed that fresh AG (1859 ± 7.07 mg/kg) had higher nitrite content than AP (1262 ± 2.12 mg/kg). Nitrites content was 506 ± 2 and 825 ± 3.5 mg/kg for AG and AP, respectively. Reheating and storage times significantly increased the conversion of nitrate to nitrite in AG and AP. Storage at 0 and 4^{o}C exhibited a significant change (P < 0.05) in nitrate and nitrite contents for both samples. Higher nitrite content was found in AP when stored at 4^{o}C and 0^{o}C. The present study indicated that storage time and temperature affected the nitrite contents in blanched AG and AP when stored in low temperatures. Apart from that reheating was also found to increase the formation of nitrite.

Keywords: Amaranthus, Nitrate, Nitrite, Reheating, Storage

ABSTRAK

Perubahan kandungan nitrat dan nitrit di dalam Amaranthus gangeticus (AG) dan Amaranthus paniculatus (AP) (daun dan batang) kesan daripada penceluran, tempoh penyimpanan (0-4 hari), suhu penyimpanan (0 dan 4º C), dan pemanasan semula dianalisis. Keputusan menunjukkan AG segar (1859 ± 7.07 mg/kg) mengandungi kandungan nitrat lebih tinggi berbanding AP (1262 ± 2.12 mg/kg). Kandungan nitrit bagi AG dan AP ialah 506 ± 2 dan 825 ± 3.5 mg/kg. Pemanasan semula dan tempoh penyimpanan meningkatkan penukaran nitrat kepada nitrit di dalam AG dan AP secara signifikan (P < 0.05). Penyimpanan pada 0 dan 4º C menunjukkan perubahan yang siginifikan pada kandungan nitrat dan nitrit bagi kedua-dua sampel. Kandungan nitrit yang lebih tinggi dalam AP apabila disimpan pada 0 dan 4^{o}C. Kajian ini menunjukkan bahawa tempoh penyimpanan dan suhu memberi kesan terhadap kandungan nitrit di dalam AG dan AP yang dicelur walaupun disimpan pada suhu rendah. Selain itu, pemanasan semula juga telah meningkatkan pembentukan nitrit.

Kata kunci: Amaranthus, Nitrat, Nitrit, Pemanasan semula, Penyimpanan

INTRODUCTION

Research on dietary intake of excess nitrates and nitrites from food has raisen the concern on their possible adverse effect on human health. Epidemiological and clinical studies showed that high level of nitrites in vegetables contribute to a significant effect in increasing the prevalence of stomach, oesophagus and bladder cancer (Bartsch et al. 1990; Umah et al. 2003). Vegetables play an important role in dietary intake of nitrate and nitrite (Kmiecik et al. 2004). It is estimated that they contribute about 85%, and 43% of the daily dietary intakes of nitrate and nitrite, respectively (Gangolli et al. 1994; Cassens, 1995; Walters, 1991).

Amaranthus, locally known as *bayam* (in Malay), is one of the most popular leafy vegetables consumed in Malaysia (Amin et al. 2006). It is one of the rich sources of nitrate among green leafy vegetables. *Amaranthus gangeticus* (AG) is referred to as *bayam merah* in Malay language. It belongs to *Amaranthaceae* family and widely consumed as green leafy vegetable in Malaysia, China and Indonesia. The very young leaves can be eaten raw as salads. It is a tender herb about 80 cm tall with few branches. Stem smooth, but longitudinally ribbed, green with red streaks, leaves alternate, elliptic shape with tapering apex. *Amaranthus paniculatus* (AP) is referred to as *bayam putih*, commonly consumed green leafy vegetable. It is a tall annual, 1.2 – 1.8 m high with stout, grooved and slightly pubescent stems, leaves simple, alternate, and broad in shape.

Several processing of vegetable such as washing, blanching, storage or freezing can contribute to the changes of nitrate and nitrite contents in foods (Bednar et al. 1991; Leszczynska et al. 2009). Recently, the consumption of leftover food has become a popular practice among Malaysian in order to prevent food wastage. Prepared foods were stored in refrigerator, and then later reheated before consumed. Many of them are unaware that reheating may cause the formation of poisonous N-nitroso compounds, which is a potential carcinogen (Ximenes, 2000; Prasad & Chetty, 2008).

The nitrate-converting enzymes produced by certain bacteria can also convert nitrates into nitrites at elevated temperatures during reheating. In theory, the longer the storage time, the higher is the concentration of nitrites (Bosch-Bosh et al. 1995). Previous studies done by researchers also showed that when spinach was kept frozen, the nitrites content remain unchanged, but when it was refrigerated, the nitrites content was seriously affected (Bosch-Bosh et al. 1995).

Thus, the aim of the study was to determine the effect of reheating, storage time and temperature on nitrate and nitrite contents in blanched *Amaranthus gangeticus* (AG) and *Amaranthus paniculatus* (AP). The information obtained through this study will benefit the public especially in educating the housewives, the correct way of handling the leftover food, particularly foods containing high nitrates content.

MATERIALS & METHODS

CHEMICALS

Phenoldisulphonic acid reagent, concentrated ammonium hydroxide, sodium nitrate, sodium nitrite, sulphonilic acid reagent and α-Naphthylamine were purchased from Sigma Co. (St. Louis, MO, USA).

MATERIALS AND SAMPLE PREPARATION

Fresh *Amaranthus paniculatus* L. (AP) and *Amaranthus gangeticus* L. (AG) was bought from five selected stalls in Pasar Borong, Selangor, Malaysia. AP and AG (1 kg each) were cleaned under running tap water and remaining water was drained off. The young leaves and tender stems were chopped into small pieces, homogenized using homogenizer and divided into two portions (raw and blanched). The raw samples (AP & AG) was directly extracted to determine the nitrate and nitrite contents. Blanching was carried out by simmering the vegetables in boiling water (100^{o}C) for 1 minute. After blanching, the remaining water was drained off and the vegetables were allowed to cool at room temperature. All blanched samples were divided into two portions to be stored at different temperatures (4 and 0^{o}C) for the following days (0, 1, 2, 3, and 4 days). Samples were reheated by blanching at 100^{o}C for 1 minute prior to the analysis of nitrates and nitrites. The nitrates and nitrites contents in blanched *Amaranthus* were determined after first and second reheating.

DETERMINATION OF NITRATES AND NITRITES

The method of nitrate and nitrites extraction was according to the method of Stopes et al.1998. Twenty five gram each of the homogenized samples was blended with 200 ml hot distilled water. The mixture was then filtered through a Whatman No. 1 filter paper, and the filtrate was used for nitrate and nitrite determinations.

DETERMINATION OF NITRATE

The filtrates of sample were analyzed for nitrate using the phenoldisulphonic acid method (Umah et al. 2003). About 20 ml of each sample filtrate was pipetted into a conical flask and the content was evaporated to dryness in water bath (100^{o}C for 15 minutes). About 2 ml of phenoldisulphonic acid reagent was added into the flask to ensure dissolutions of all the solid contents. It was then diluted with 20 ml distilled water and 6 ml concentrated ammonium hydroxide, followed by stirring. Absorbance was read at 410 nm using a spectrophotometer (Shimadzu 160 A model, Japan) against a blank prepared with distilled water. A standard nitrate curve was prepared using 50 mg/L stock sodium nitrate solution.

DETERMINATION OF NITRITE

The diazotization method (Umah et al. 2003) was used in the determination of nitrite content. About 20 ml of each sample filtrate were pipetted into a conical flask. Sulphanillic acid reagent (0.5 ml) was added to each flask and left to stand for 2 to 8 minutes. a-Naphthylamine (0.5 ml) was then added and allowed to stand for about 2 hours for the full development of the characteristic pinkish-red color. The absorbance was measured at a wavelength of 543 nm on the spectrophotometer against distilled water blank. A standard nitrite curve was prepared using 50 mg/L stock sodium nitrite solution.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Science (SPSS) version 15. Results were expressed as mean ± standard deviation (SD). One way ANOVA was used to analyze the means differences among the samples under different treatments. The significant level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Amaranthus gangeticus (AG) contained more nitrates content (1859 ± 7.07 mg/kg) when compared to AP (1262 ± 2.12 mg/kg). The results of the present study for both *Amaranthus* were in the same range of nitrate content as reported earlier by Anjana et al. (2007) and Reinik (2007). Nitrites content in raw AG and AP were 506 ± 2.12 mg/kg and 825 ± 3.53 mg/kg respectively. There was a significant difference ($P < 0.05$) between the nitrites content in both species. Therefore, the trend for nitrates content was AG > AP while AG < AP for nitrites content.

CHANGES IN NITRATES AND NITRITES CONTENTS OF *A. GANGETICUS* AND *PANICULATUS* DURING STORAGE AT 4ºC

In this study, the effects of different storage time and temperature on nitrate and nitrite contents in blanched *Amaranthus* species were studied over a period of four days. Significant differences ($P < 0.05$) were found in the means of nitrate and nitrite contents of AG and AP at different storage temperature and storage days. The loss of nitrates in *Amaranthus* stored for 0, 1, 2, 3, and 4 days at 4ºC ranged from 4-49% for AG and from 0.2-46% for AP (Figure 1). This indicated that the longer the storage time, the higher the conversion of nitrate to nitrite. As a result, nitrite content increased but, conversely nitrate content decreased. Nitrite content showed an increasing trend toward storage time with the lowest content at day 0 followed by day 1, 2, 3, and day 4. The increment of nitrites ranged from 21-70% for AG and 4-54% for AP (Figure 2). Therefore, it is advisable that *Amaranthus* should never be kept under refrigeration for more than 2 days after cooking as the increment of nitrites content was almost fifty percent. Previous studies showed that microbiological reduction of nitrates and increment in nitrites takes place more rapidly when *Amaranthus* was stored under refrigeration (Domanska-Blicharz et al. 2004). Bosch-Bosch et al. (1995) in their study showed that *Amaranthus* stored (under refrigeration) for 2 days affected the nitrite levels, however, the contents become significant after 4 days of storage under refrigeration.

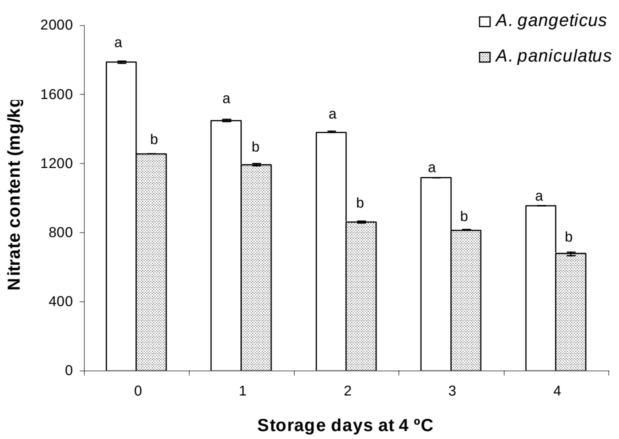

FIGURE 1. Nitrates content in *Amaranthus gangeticus* and *Amaranthus paniculatus* at 4ºC. The value was expressed as mean ± SD. (n = 3). Different alphabet indicated significant difference ($P < 0.05$) between the two types of *Amaranthus* species

CHANGES IN NITRATES AND NITRITES CONTENTS OF *A. GANGETICUS* AND *A. PANICULATUS* DURING STORAGE AT 0ºC

The nitrate contents decreased from 1787 ± 4.95 mg/kg at day 0 to 966 ± 7.07 on day 4 in AG. A similar trend was also noticed in AP wherein the nitrate content decreased from 1259 on day 0 to 1056 ± 2.83 on day 4 (Figure 3). Storage at 4ºC exhibited highest nitrate losses compared to 0ºC, since nitrate converting bacteria are less active, thus the conversion of nitrate to nitrite was much slower (Prasad & Chetty 2008; Schuddeboom 1993). Increment of nitrites was slightly lower compared with storage at 4ºC and the increment in nitrite content varied from 22-63% for AG and 4-45% in AP (Figure 4). During storage, the nitrite content was reported to increase, especially at high temperature, due to reduction of nitrate to nitrite by the enzyme *reductase* present in bacteria (*E.Coli*) commonly found in water used for cooking (Schuddeboom 1993). Similar increment in nitrite content of carrot juice was reported by Hall et al. (1977) during storage at 4ºC. Thus, storage at different temperatures (0 & 4ºC) exhibited significant changes in the means of nitrate and nitrite contents for AG and AP. Higher nitrite content was found in AP when stored at 4 and 0ºC than AG. This is because of the presence of carotenes in AG which might act as chemopreventive phytochemicals which could inhibit the formation of N-nitroso compounds (Huzaimah et al. 2004).

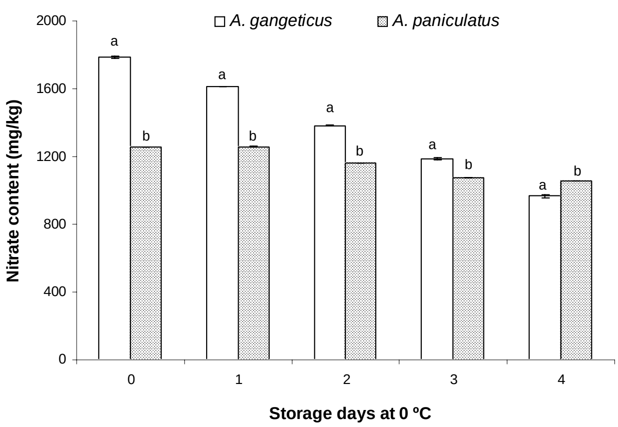

FIGURE 3. Nitrates content in *Amaranthus gangeticus* and *Amaranthus paniculatus* at 0ºC. The value was expressed as mean ± SD. (n = 3). Different alphabet indicated significant difference ($P < 0.05$) between the two types of *Amaranthus* species

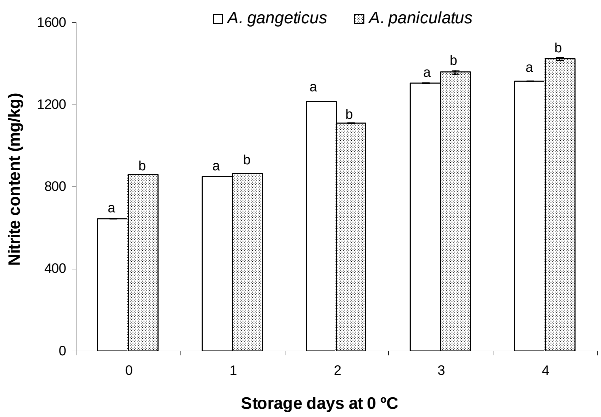

FIGURE 4. Nitrites content in *Amaranthus gangeticus* and *Amaranthus paniculatus* at 0°C. The value was expressed as mean ± SD. (n = 3). Different alphabet indicated significant difference (P < 0.05) between the two types of *Amaranthus* species

CHANGES IN NITRATES AND NITRITES CONTENTS OF *A. GANGETICUS* AND *A. PANICULATUS* DURING HEATING AND REHEATING CONDITIONS

Additionally, the effect of heating and reheating on the content of nitrate and nitrite of *Amaranthus* species during storage at 0 and 4°C was also evaluated (Table 1 & 2). The nitrate content of raw AG at day zero was 1859 ± 7.07 mg/kg. After first reheating, the nitrate content decreased to 1787 ± 4.95 mg/kg and further decreased to 1610 mg/kg after second reheating. The decreasing order of nitrates content was as follows: raw > first reheating > second reheating. After 4 days of storage, the highest nitrate loss under 4 and 0°C was found in second reheating while the lowest nitrate loss was found on day zero in AG and AP after first reheating (Table 1). However, the highest nitrite accumulation was on fourth day for both storage at 4°C and 0°C whereas the lowest nitrite accumulation was at day zero (Table 2). A significant difference (P < 0.05) between the nitrates contents in first and second reheating may be due to the solubility of nitrates into water during blanching as reported by Reinik (2003). Storage for 2 days under refrigeration did affect the nitrite levels. The contents become significant after 4 days under refrigeration (Bosch-Bosch et al. 1995). Nitrates transform themselves into nitrites and this process also could be inhibited in low temperature (Schuddeboom 1993). Moreover, Hall et al. (1977) suggested that nitrites content changed over period of 6 hours with increase in temperatures. Hill (1996) also reported that the rate of nitrites content increased with temperature over a 6 hour period. This was true for both *Amaranthus* when the samples were reheated again for second times after storage for six hours. Hence, reheating and storage times had significantly increased the conversion of nitrate to nitrite in AG and AP.

Leafy vegetables are known to provide a significant amount of nitrates in our diet. Nitrates and nitrites amounts of foods are of great important regarding the consumers' health because excessive nitrate accumulation can occur in some roots and leafy vegetables (Ozcan & Akbulut 2007).

According to Keeton et al. (2009), vegetables having the highest nitrate concentrations (1,000 – >2,500 ppm) include lettuce, spinach, red beets, radishes, celery, parsley turnip greens; mid-range (500-1,000 ppm); vegetables include cabbage, turnip; low-range (200-500 ppm) examples are broccoli, carrots, cucumbers, cauliflower, pumpkin, egg plant, green onions, melon and very low-range (< 200 ppm) vegetables include potato, peppers, sweet potatoes, tomatoes. From the results obtained, we can classify the vegetables used in the current research (AP and AG) as high in nitrate content. No literature data on nitrite content (high, medium and low level) is available.

According to Joint FAO/WHO Expert Committee on Food Additives in 2002, the Acceptable Daily Intake (ADI) values are 3.7 mg/kg body weight for nitrate and 0.07 mg/

TABLE 1. Changes in content of nitrates in *Amaranthus gangeticus* and *Amaranthus paniculatus* during reheating, storage time and storage temperature

Leafy vegetable	Storage Days	First reheating Mean ± S.D (mg/kg)		Second reheating Mean ± S.D. (mg/kg)	
		Refrigeration (4 °C)	Frozen (0 °C)	Refrigeration (4 °C)	Frozen (0 °C)
A. gangeticus	0	1787 ± 4.95	1787 ± 4.95	1610 ± 0.00	1610 ± 0.00
	1	1450 ± 7.07 (-19%)	1610 ± 0.00 (-10%)	1437± 0.00 (-11%)	1440 ± 0.00 (-11%)
	2	1382 ± 3.54 (-33%)	1383 ± 3.53 (-33%)	1193 ± 8.49 (-26%)	1244 ± 0.00 (-23%)
	3	1119 ± 0.00 (-38%)	1189 ± 7.07 (-34%)	1103 ± 1.41 (-32%)	1157 ± 8.48 (-29%)
	4	954 ± 0.00 (-57%)	966 ± 7.070 (-56%)	940 ± 7.78 (-42%)	936 ± 6.36 (-42%)
A. paniculatus	0	1259 ± 0.00	1259 ± 0.00	1258 ± 2.12	1258 ± 2.12
	1	1193 ± 8.49 (-8%)	1258 ± 3.54 (-1%)	1037 ± 7.07 (-18%)	1191± 0.00 (-06%)
	2	863 ± 6.36 (-32%)	1165 ± 0.03 (-8%)	852 ± 4.95 (-33%)	1114 ± 0.00 (-12%)
	3	813 ± 2.83 (-36%)	1073 ± 0.00 (-15%)	775 ± 4.24 (-39%)	1067 ± 2.83 (-16%)
	4	679 ± 9.19 (-54%)	1056 ± 2.83 (-17%)	494 ± 0.00 (-61%)	717 ± 0.07 (-44%)

Values in the brackets denote a decrease in percentage of nitrates compared to day zero of storage.

TABLE 2. Changes in content of nitrites in *Amaranthus gangeticus* and *Amaranthus paniculatus* during reheating, storage time and storage temperature

Leafy vegetable	Storage Days	First reheating Mean ± S.D (mg/kg)		Second reheating Mean ± S.D. (mg/kg)	
		Refrigeration (4 °C)	Frozen (0 °C)	Refrigeration (4 °C)	Frozen (0 °C)
A. gangeticus	0	644 ± 0.00	644 ± 0.00	673 ± 0.00	673 ± 0.00
	1	1286 ± 0.00 (+196%)	850 ± 0.00 (+131%)	1388 ± 0.01 (+206%)	1191 ± 7.07 (+176%)
	2	1305 ± 1.41 (+202%)	1215 ± 2.12 (+188%)	1392 ± 0.00 (+206%)	1302 ± 0.00 (+193%)
	3	1393 ± 0.00 (+216%)	1305 ± 1.41 (+202%)	1572 ± 0.02 (+226%)	1325 ± 0.00 (+196%)
	4	1636 ± 1.41 (+254%)	1316 ± 0.00 (+204%)	1668 ± 0.00 (+247%)	1384 ± 0.00 (+205%)
A. paniculatus	0	858 ± 0.00	858 ± 0.00	944 ± 0.00	944 ± 0.00
	1	1529 ± 6.36 (+178%)	866 ± 0.00 (+100%)	1570 ± 9.90 (+166%)	1105 ± 0.02 (+117%)
	2	1602 ± 1.41 (+186%)	1112 ± 0.00 (+129%)	1628 ± 0.00 (+172%)	1165 ± 6.36 (+123%)
	3	1645 ± 8.49 (+191%)	1358 ± 7.07 (+158%)	1689 ± 3.54 (+178%)	1375 ± 4.95 (+145%)
	4	1729 ± 7.07 (+201%)	1423 ± 7.78 (+165%)	1785 ± 0.01 (+189%)	1491 ± 0.01 (+157%)

Values in the brackets denote an increase in percentage of nitrites compared to day zero of storage

kg b.w. for nitrite (Anonymous 2008). Approximately 5% of all dietary nitrates are reduced to nitrites in saliva and gastrointestinal tract. However, nitrites being highly unstable as compared to nitrates are easily metabolized within the digestive tract to N-nitroso compounds (Prasad & Chetty 2008). Greater nitrite content thus could increase the likelihood of endogenous nitrosamine reactions, which in turn may lead to a greater risk of cancer. The main concern for the public health is the link between nitrates and stomach cancer. The contents of nitrates and nitrites in raw *Amaranthus* were significantly different compared to blanched *Amaranthus*. AG had higher nitrates content compared to AP. When compared between both samples, AG was reported to accumulate large amount of nitrate due to their low nitrate reductase activity.

CONCLUSIONS

The present study indicated that storage time and temperature affected the conversion of nitrate to nitrite in blanched AG and AP when stored in refrigeration (4°C) and frozen (0°C). In addition, reheating of blanched *Amaranthus* after stored at low temperature also affected the formation of nitrite contents. Second reheating increased nitrite contents even more compared to first reheating. Besides that, blanching also contributed to significant decreased in nitrate contents. In conclusion, it is advisable that fresh cooked *Amaranthus* must be consumed as soon it is cooked and should not be stored for more than two days. Further work on the accumulation of oxalates in *Amaranthus* during different storage time and temperature is needed to be investigated.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of the laboratory staffs from the Department of Nutrition and Dietetics, Universiti Putra Malaysia.

REFERENCES

Amin, I., Norazidah, Y. & Emmy, H.I. 2006. Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chem*. 94: 47-52.

Anjana, Shahid, U., Muhammad, I. & Abrol, Y.P. 2007. Are nitrate concentrations in leafy vegetables within safe limits? *Current Sci*. 92: 355-360.

Anonymous. 2008. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. *The EFSA Journal.* 689, 1-79 (online). http://www.efsa.europa.eu/en/efsajournal/doc/contam_ej_689_nitrate_en.pdf

Bartsch, H., Ohshima, H., Shuker, D.E.G., Pignatelli, B. & Calmels, S. 1990. Exposure of humans to endogenous N-nitroso compounds: Implications in cancer etiology. *Mutation Res*. 238: 255-267.

Bednar, C.M., Kies, C. & Carlson, M. 1991. Nitrate–nitrite levels in commercially processed and home processed beets and spinach. *Plt. Foods for Human Nutr*. 41: 261-268.

Bosch-Bosch, N., Garcia-Mata, M., Penuela, M.J., Ruiz-Galan, T. & Lopez-Ruiz, B. 1995. Determination of nitrate levels in refrigerated and frozen spinach by ion chromatography. *J. Chrom. A.* 706: 221-228.

Cassens, R. 1995. Use of sodium nitrite in cured meats today. *Food Technol*. 50: 72-80.

Domanska-Blicharz, K., Michalski, M. & Kowalski, B. 2004. Effect of different storage conditions on nitrates and nitrites in polish edible offals processed meat products. Influence on N-nitrosamine content. *Bull. Vet. Ins. Pulawy*. 48: 63-68.

Gangolli, S.D., van den Brandt, P.A., & Feron, V.J. 1994. Nitrate, nitrite and *N*-nitroso compounds. *Eur. J. Pharm. Env. Tox. & Pharm*. Sec. 292: 1-38.

Hall, C.B., Hicks, J.R. & Stall, R.E. 1977. Nitrates in inoculated carrot juice as a function of nitrate content and temperature. *J. Food Sci*. 42: 549-550.

Hill, M.J. 1996. Nitrates and nitrites in food and water. Woodhead Publishing Limited. New York. pp. 21-26.

Huzaimah, A.S., Asmah, R., Maznah, I., Rozita, R. & Susi, E. 2004. Potential anticancer effect of red spinach (*Amaranthus gangeticus*) extract. *Asia Pac. J. Clin. Nutr.* 13: 396-400.

Keeton, J.T., Osburn, W.N., Hardin, M.D., Bryan, N.S. & Longnecker, M.T. 2009. A national survey of nitrite/nitrate concentration in cured meat products and non-meat foods available in retail. www.amif.org/ht/a/GetDocumentAction/i/52741

Kmiecik, W., Lisiewska, Z. & Slupski, J. 2004. Effects of freezing and storage of frozen products on the content of nitrate, nitrites, and oxalates in dill (*Anethum graveolens L.*). *Food Chem.* 86: 105-111.

Leszczynska T., Filipiak-Florkiewicz, A., Cieslik E., Sikora, E. & Pisulewski, P.M. 2009. Effects of some processing methods on nitrate and nitrite changes in cruciferous vegetables. *J. Food Comp. & Anal.* 22: 315-321.

Ozcan, M.M. & Akbulut, M. 2007. Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea. *Food Chem.* 106: 852-858.

Prasad, S. & Chetty, A.A. 2008. Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing. *Food Chem.* 106: 772-780.

Reinik, M. 2003. Naturally occurring nitrates and nitrites in food in bioactive substances in foods – natural toxicants and heat processing contaminants. Doctoral dissertation, University of Tartu, Estonia. pp. 1-126.

Schuddeboom, L.J. 1993. Nitrates and nitrites in foodstuff. *Council Europe Press*. Ministry of Welfare, Health and Culture, Netherlands. pp. 1-124.

Stopes, C., Woodward, L., Frorde, G. & Vogtman, H. 1988. The nitrate content of vegetables and salads crops offered to consumer. *Biological Agri.* 5: 215-221.

Umah, Joy, A. Ketiku, A.O. & Sridhar, M.K.C. 2003. Nitrate, nitrite and ascorbic acid content of commercial and home-prepared complementary, infant foods. *African J. Biomed. Res*. 6: 15-20

Walter, C. 1991. Nitrate and nitrite in foods. In *Nitrate and Nitrites in Food and Water* (M. Hill, Ed), Ellis Harwood, New York. pp. 93-107.

Ximenes, M.I.N. 2000. Polarographic determination of nitrate in vegetables. *Talanta*. 51: 49-56.

Amin Ismail, Chew Sc, K. Nagendra Prasad
Department of Nutrition and Dietetics
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia

Laboratory of Analysis and Authentication
Halal Products Research Institute
Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia

Yang Bao
South China Botanical Garden
Chinese Academy of Sciences
Guangzhou 510650
The People's Republic of China

Corresponding author: Amin Ismail
Email address: amin@medic.upm.edu.my
Tel: 603 89472435; Fax: 603 89472459

Received: August 2010
Accepted for publication: December 2010