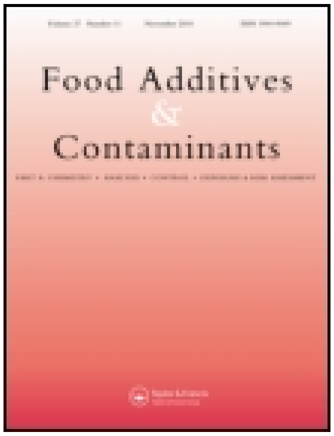

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Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems

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Abstract

The nitrate content of leafy vegetables (watercress, lettuce and arugula) produced by different agricultural systems (conventional, organic and hydroponic) was determined. The daily nitrate intake from the consumption of these crop species by the average Brazilian consumer was also estimated. Sampling was carried out between June 2001 to February 2003 in Campinas, São Paulo State, Brazil. Nitrate was extracted from the samples using the procedure recommended by the AOAC. Flow injection analysis with spectrophotometric detection at 460 nm was used for nitrate determination through the ternary complex $FeSCNNO^{+}$. For lettuce and arugula, the average nitrate content varied ($p < 0.05$) between the three agricultural systems with the nitrate level in the crops produced by the organic system being lower than in the conventional system that, in turn, was lower than in the hydroponic system. For watercress, no difference ($p < 0.05$) was found between the organic and hydroponic samples, both having higher nitrate contents ($p < 0.05$) than conventionally cultivated samples. The nitrate content for each crop species varied among producers, between different parts of the plant and in relation to the season. The estimated daily nitrate intake, calculated from the consumption of the crops produced by the hydroponic system, represented 29% of the acceptable daily intake established for this ion.

Keywords: *Nitrate, conventional agriculture, organic agriculture and hydroponic agriculture*

Introduction

The concentration of nitrate in vegetables varies according to the species, the varieties and the availability of this nutrient to the plant, as well as due to environmental factors such as light intensity and temperature. In addition, the nitrate content depends on the harvest period, the agricultural system, the maturation stage and the part of the plant (Maynard et al. 1976; Van der Boon et al. 1990; Steingröver et al. 1993; Lyons et al. 1994; McCall and Willumsen 1998; Amr and Hadidi 2001).

In conventional cultures, the vegetables are grown in soil with an adequate supply of nutrients and water. For good production, chemical substances are often used such as mineral fertilisers and pesticides for insect and fungal control. On the other hand, the organic agricultural system avoids the use of chemical compounds such as fertilisers and pesticides. The organic production system adopts the practice of cultivation rotation, organic residue recycling, and biological handling and control. In this agricultural technique, the intent is to maintain microbial life in the soil in order to supply plants with nutrition and health. Organic production presents a great advantage compared with conventional agriculture due to its beneficial impact on the environment.

Hydroponics is a system of cultivation of plants in water, in the absence of soil, where the nutrients required for plant growth are provided through the water by means of a nutrient solution (Resh 1995). The main advantages of the system are the production of homogenous and high-quality vegetables, harvest anticipation, a reduction of the appearance of plagues and/or diseases, and production throughout the entire year.

The employment of nitrogenous chemical fertilisers usually used in conventional and hydroponic

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cultivation may lead to a higher nitrate content in some vegetables (Lyons et al. 1994). Several studies also indicate that vegetables cultivated through the conventional method show a higher nitrate content when compared with vegetables cultivated through organic agriculture (Woese et al. 1997).

The nitrate content in food, in particular vegetables, associated with higher consumption is of great interest due to possible adverse effects to human health. In most diets, vegetables generally contribute over 70% of the total nitrate ingested. In particular, leafy vegetables have a higher contribution to this exposure due to their high nitrate content (Walker 1990). In general, nitrate is considered to be of low toxicity. However, when reduced to nitrite, it may represent a high risk to human health. In fact, nitrate is easily reduced to nitrite, which may interact with haemoglobin, affecting oxygen transport and leading to methaemoglobinaemia. Nitrite also reacts with secondary and tertiary amines forming N-nitroso compounds (Walker 1990), which are associated with a high risk of stomach, liver and oesophagus carcinomas (Siddiqi et al. 1992; Wu et al. 1993; Mitacek et al. 1999; Kim et al. 2002). Nonetheless, diets rich in vegetables have been associated with a reduction in the risk of cancer due to the presence of antioxidants and other anticarcinogenic substances (WHO 1996).

In relation to the toxicological limit, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) established acceptable daily intakes (ADIs) of 0–3.7 and 0–0.07 $mg\,kg^{-1}$ body weight for nitrate (expressed as nitrate ion) and nitrite (expressed as nitrite ion), respectively (WHO 2003). With regards to the nitrate content in vegetables, the European Union established for lettuce produced in green houses maximum levels of 3500 and 4500 $mg\,kg^{-1}$ (fresh weight) for the summer and winter seasons, respectively. The maximum level for lettuce produced in open fields is 2500 and 4000 $mg\,kg^{-1}$ for the summer and winter seasons, respectively. In addition, the nitrate concentration in 'iceberg'-type lettuce produced in open fields should not exceed 2000 $mg\,kg^{-1}$; and for that produced in greenhouses, 2500 $mg\,kg^{-1}$ (EC 2001). It is worth indicating that Brazil does not have legislation regarding the presence of nitrate in vegetables. Most of the available data related to nitrate in vegetables also have been established in cultures harvested in temperate climates.

The aim of the present paper was to evaluate the nitrate content in watercress, lettuce and arugula produced by different agricultural systems (conventional, organic and hydroponic) in the Campinas region, SP, of Brazil. Nitrate determination was performed by flow injection analysis (FIA) with spectrophotometric detection. The samples were obtained from different producers, cultivated during different seasons of the year. The daily nitrate intake due to the consumption of these crops by the average Brazilian consumer was also estimated.

Materials and methods

Chemicals

All chemicals used were of analytical grade or equivalent, all purchased from Merck (Rio de Janeiro, RJ, Brazil). Solutions were prepared with purified water from a Milli-Q Plus System (Millipore, Bedford, MA, USA).

Samples

Samples of watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek), lettuce (*Lactuca sativa* L.) and arugula or rocket salad (*Eruca vesicaria* ssp. *sativa* (P. Mill.) Thellung) produced by conventional, hydroponic and organic agricultural systems were acquired directly from producers ($n = 161$), markets (9) and supermarkets (8) in Campinas, totalling 178 samples. The selection of these vegetables was due to their availability from all three agricultural systems. All organic samples analysed had a certification stamp awarded by the Campinas Natural Agriculture Association (www.anc.org.br/) or by the Mokiti Okada Foundation (www.fmo.org.br/cpmo/agricultura.asp). All the hydroponic lettuce was grown under cover. Random sampling was carried out during June 2001–February 2003.

Sample preparation

The edible parts of lettuce (90 g), watercress (60 g) and arugula (60 g) were washed with water, dried with filter paper and triturated in a Waring blender with water in a 1:1 (w/w) proportion until a homogeneous mass was attained. Approximately 5 g of the homogeneous mass was weighted and stored at -18°C until analysis.

The nitrate was extracted from the vegetable matrix with water at 70°C according to the procedure recommended by the AOAC (1997). For this purpose 40 ml deionized water were added to the 5 g homogenized sample and the solution was maintained for 15 minutes in a water bath at 70°C. The sample was cooled to room temperature, transferred to a 100-ml volumetric flask and the volume completed with deionized water. The extracts were filtered through filter paper (Whatman No. 4), and to obtain the desired concentrations of nitrate, the filtrate was diluted with water before injection into the FIA system. For each determination, three replicates were performed.

Nitrate determination by the FIA method

The FIA system was composed of a four-channel Ismatec peristaltic pump (Glattbrugg, Switzerland) fitted with Tygon tubing (1.2 mm i.d.). Sample injection was performed in a three-section manual commutator made of acrylic, with two fixed side bars and a sliding central bar that was moved between sampling and injection. The absorbance was measured at 460 nm with an FEMTO spectrophotometer (Model 432) (Sao Paulo, Brazil) equipped with a glass flow cell with a 10-mm optical pathway. The transient absorbance signals were monitored by an Intralab two-channel strip-chart recorder (Sao Paulo, Brazil). For more information, see Andrade et al. (2003).

Food consumption data

The source of data on food consumption used was the *Survey on Household Budgets* conducted by the Brazilian Institute of Geography and Statistics, during 2002 and 2003 in Brazilian metropolitan regions (IBGE 2003). A description of the procedure adopted by the IBGE has been reported by Baunwart and Toledo (2001).

Statistical analysis

The number of samples (*n*) analysed for each producer of each cultivar was determined by the following Equation (Cochran 1977):

$$\left[\frac{(\max - \min/6)}{(\max + \min/2)}\right]^2 \leq n \leq 400\left[\frac{(\max - \min/4)}{(\max + \min/2)}\right]^2$$

where 'max' is the maximum nitrate content and 'min' is the minimum nitrate content determined in five to ten samples from each crop species (arugula, watercress and lettuce) obtained from a single producer. The minimum number of samples calculated for analysis was three samples from each producer. The values used to calculate the number of samples were: arugula (maximum = 7759, minimum = 4641), watercress (6160, 4194) and lettuce (3693, 2172).

The comparison of the nitrate content in the crops produced under different agricultural systems (organic, conventional and hydroponic), from different producers or cultivated at different seasons of the year was performed through ANOVA and a *t*-test. The statistical tests were performed using the GraphPad Software Prism program, version 2.01 (GraphPad Software, Inc.).

Table I. Nitrate content in lettuce, watercress and arugula produced by different agricultural systems.

Crop species	Agricultural system	n	$NaNO_3$ content ($mg\,kg^{-1}$): Average $\pm s$ ($mg\,kg^{-1}$)	Range ($mg\,kg^{-1}$)
Lettuce	Organic	21	$818^a \pm 489$	115–1852
	Conventional	24	$1303^b \pm 430$	677–2179
	Hydroponic	37	$2983^c \pm 554$	1842–4022
Arugula	Organic	16	$4073^a \pm 1260$	2160–5670
	Conventional	19	$5377^b \pm 1428$	3726–8268
	Hydroponic	18	$8243^c \pm 982$	6461–9703
Watercress	Organic	15	$5180^a \pm 689$	3340–5926
	Conventional	6	$1234^b \pm 738$	296–2388
	Hydroponic	22	$4873^a \pm 874$	2009–6160

n, Number of samples; *s*, estimated standard deviation; different letters (a–c) indicate the difference ($p < 0.05$) between agricultural systems for each crop species.

Results and discussion

The FIA method was based on nitrate determination through the ternary complex $FeSCNNO^+$, which absorbs at 460 nm, formed from NO, Fe^{2+} and SCN^- in an acid medium. The method was previously developed and in-house validated. It is described by Andrade et al. (2003). For lettuce, arugula and watercress, the limit of detection (LOD) and the limit of quantification (LOQ) for nitrate were 6.0 and 20 $mg\,kg^{-1}$, respectively. The recovery rate of nitrate in the matrices varied from 93 to 110%; and the variability in determination (between-run precision) over 5 days presented a relative estimated standard deviation of 5.5%.

Nitrate content in the leafy vegetables

The nitrate content in watercress, arugula and lettuce varied with the vegetable and the agricultural system. The lowest nitrate contents for lettuce and arugula were obtained in the organic agricultural system, whereas for watercress the conventional system provided the lowest nitrate levels (Table I).

It is worth emphasizing that comparative studies have demonstrated a lower nitrate concentration in vegetables cultivated through organic agriculture in relation to the conventional one (Gento 1994; Woese et al. 1997; Yordanov et al. 2001; Bourn and Prescott 2002). However, some studies did not indicate much difference (Péres-Llamas et al. 1996; Malmauret et al. 2002) and several others reported the opposite (Gent 2002; Martin and Restani 2003).

In the present study, for lettuce and arugula a difference ($p < 0.05$) between the three agricultural systems was verified. The vegetables cultivated through the organic system showed an average nitrate content lower than in plants from conventional cultivation, and this was lower than in vegetables cultivated through hydroponics. These results are in agreement with those reported by Miyazawa et al. (2001). None of the lettuce samples from organic and conventional agricultural systems presented nitrate concentrations above the maximum level established by European Union legislation. Nevertheless, for the hydroponic system, 5% of the samples cultivated in the summer contained nitrate levels above the legal limit established by European Union legislation (3500 mg kg^{-1}) (EC 2001). With regard to samples of watercress and arugula, there is no legislation for nitrate content.

The nitrogen supply is the most important nutritional factor that affects the accumulation of nitrate in vegetables. The usual effect is that as the level of nitrogen increases, the concentration of nitrate in the vegetables also increases (Maynard et al. 1976). Nitrate, phosphate and potassium present in mineral fertilisers are in forms available to plants. Organic fertilisers contain nutrients in the form of nitrogenous salts and organic compounds, which are gradually released as the result of the action of microorganisms present in the soil, thus becoming available to the plants (Saffron 1998). This explains the results observed for samples of hydroponic lettuce and arugula, in which nitrate is completely available, enabling efficient absorption from the nutritive solution and, consequently, a higher nitrate content in those plants in relation to the other cultivation systems.

On the other hand, no difference ($p < 0.05$) between organic and hydroponic agricultural systems was verified for watercress, and the nitrate content present in samples from those agricultural systems was higher ($p < 0.05$) than in conventionally cultivated watercress.

The nitrate content in all crop species analysed varied in relation to the different parts of the leaf (Table II). Generally, there was a higher nitrate level in stalks in relation to the rest of the leaf. The different capacity of accumulating nitrate could be related to the different localization of the activity of the enzyme nitrate reductase, as well as to the different absorption and transportation degrees in plants (Maynard et al. 1976).

Table II. Nitrate content in different parts of the leaf.

Crop species	Agricultural system	$NaNO_3$ content $\pm s$ (mg kg^{-1}) Stalk (S)	Leaf without stalk (LWS)	Relation: S/LWS
Lettuce	Conventional	1470 ± 118	577 ± 74	2.55
	Hydroponic	3927 ± 198	3084 ± 93	1.27
Arugula	Organic	5057 ± 116	1002 ± 53	5.05
	Conventional	7160 ± 147	2865 ± 84	2.50
Watercress	Hydroponic	7126 ± 191	3750 ± 140	1.90
	Organic	8187 ± 159	3158 ± 71	2.59

s, Estimate standard deviation ($n = 3$).

Seasonal influence on the nitrate content

In general, the nitrate content in lettuce and arugula cultivated by conventional and organic systems was higher during autumn and winter than during spring and summer, the difference being significant ($p < 0.05$) when comparing the winter and summer seasons. The nitrate content in plants varies according to the cultivation season, since the light period and the luminous intensity influence the activity of the enzyme nitrate reductase. In short, in photoperiods (winter) where plants are submitted to low luminous intensity, nitrate content trends to be higher (Maynard et al. 1976; Van der Boon et al. 1990). Nevertheless, the nitrate content in hydroponic lettuce and arugula was not influenced by the season ($p < 0.05$). With regards to watercress, for samples produced by organic and hydroponic agricultural systems, no difference ($p < 0.05$) in the average nitrate content was verified between seasons. (Table III).

Although in this study no difference ($p < 0.05$) in the nitrate content in hydroponic samples cultivated during different seasons was verified, some studies carried out in Europe have demonstrated that the nitrate levels in vegetables cultivated in the winter are higher than in vegetables cultivated in the summer (MAFF 1998, 2004; Petersen and Stoltze 1999; Ysart et al. 1999). This disagreement in the results may be due to the different environmental conditions, since hydroponics cultivation is performed in greenhouses under controlled conditions.

Estimate of the average per capita nitrate daily intake

The estimate of daily nitrate intake by the Brazilian consumer was based on the per capita yearly average intake of lettuce and watercress provided by the survey carried on by the IBGE (2003). Since no data regarding arugula intake are available, it was considered similar to the watercress intake. According to the available data, the daily watercress intake ranged from 0.01 to 0.9 g and for lettuce from 0.1 to 12.5 g in the larger state capitals.

The nitrate intake estimated for each crop species produced by the three agricultural cultivation systems is presented in Table IV. Among the crop species, the hydroponic agricultural system was

Table III. Influence of the type of agricultural cultivation system and season on the average nitrate content in lettuce, arugula and watercress.

Crop species	Agricultural system	$NaNO_3$ content $\pm s$ ($mg\,kg^{-1}$)			
		Autumn	Winter	Spring	Summer
Lettuce	Organic	550 ± 275	1426 ± 294	728 ± 441	1023 ± 674
	Conventional	2033 ± 182	1718 ± 414	1153 ± 361	1198 ± 122
	Hydroponic	2765 ± 709	3158 ± 260	–	3110 ± 447
Arugula	Organic	5276 ± 313	4718 ± 100	3911 ± 695	2246 ± 64
	Conventional	7601 ± 444	4866 ± 257	4709 ± 231	4282 ± 446
	Hydroponic	8330 ± 1083	–	–	8015 ± 698
Watercress	Organic	–	5253 ± 642	4790 ± 1009	5441 ± 254
	Hydroponic	5145 ± 621	–	5237 ± 639	4521 ± 326

s, Estimate standard deviation.

Table IV. Estimated maximum daily ingestion of nitrate ion from watercress, lettuce and arugula intake.

Crop species	Daily intake* (g)	Estimate of nitrate intake (mg)		
		Organic	Conventional	Hydroponic
Lettuce	12.5	23.5	27.2	50.3
Arugula	0.9	5.1	9.2	8.7
Watercress	0.9	5.3	2.1	5.5
Total	14.3	33.9	38.5	64.5
ADI (%)**	–	15.3	17.3	29.0

*Data from IBGE (2003).
**ADI (3.7 $mg\,kg^{-1}$ body weight per day, expressed as nitrate ion) was multiplied by the average weight of a 60-kg adult = 222 mg.

responsible for the highest contribution of nitrate intake, 29% of the ADI established for this ion (WHO 2003), which indicate that for the average Brazilian consumer, it is unlikely to exceed the nitrate ADI from the consumption of lettuce arugula and watercress.

Conclusions

The results indicate that lettuce and arugula cultivated by hydroponics had nitrate contents higher than those crops cultivated by the conventional system, which in turn had higher nitrate contents than those from the organic system. Nevertheless, watercress had no differences in nitrate content between organic and hydroponic agricultural systems. These values were higher than conventionally cultivated watercress.

The nitrate content of each crop species varied among the different producers. It was higher in the stalks in relation to the other parts of the leaf. Organic watercress and all the crop species cultivated by hydroponic agricultural systems also showed a homogeneous nitrate content between the different seasons. On the other hand, organic and conventional lettuce and arugula showed a higher nitrate content in the winter in relation to the summer.

The ADI for nitrate, calculated from the Brazilian consumption of the crop species produced by the hydroponic agricultural system, represented 29% of the ADI established for this ion. Consequently, it is unlikely that the nitrate ADI will be exceeded by the average Brazilian consumer of watercress, lettuce and arugula. Nonetheless, since food consumption data for risk assessment should be based on the intakes by individuals, it is recommended that research be conducted on actual food intake by Brazilian consumers.

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