INTRODUCTION

During the plant growth and development, nitrate (NO$_3^-$) is one of the most abundant and important sources of nitrogen, independent of the growing system (Cardenas-Navarro et al., 1999; Iammarino et al., 2013). In plants, nitrate concentration depends on its absorption and assimilation. Nitrate is absorbed in the root and its excess is accumulated in storage organs (Bóbics et al., 2016). Therefore, large amounts of nitrate in plants, especially in leafy vegetables, are commonly observed (Alexander et al., 2008; Bian et al., 2018; Cardenas-Navarro et al., 1999; Iammarino et al., 2013).

The consumption of vegetables as sources of phytochemicals in the human diet has been related with health-promoting properties (Bian et al., 2018; Chan, 2011). However, as most of leafy vegetables have a great ability to accumulate nitrate especially in its leaves, they are the main contributors in human intake of this compound (Alexander et al., 2008; Bian et al., 2018; Bóbics et al., 2016; Weitzberg and Lundberg, 2013). It is estimated that 60 to 80% of the daily human consumption of nitrate are from vegetables (Suresh et al., 2018; Weitzberg and Lundberg, 2013). Nitrate levels in vegetables usually higher than 700 mg kg$^{-1}$ can result in the increasing of harmful effects on human health (Bruning-Fann and Kaneene, 1993), such as methemoglobinemia and cancers (Alexander et al., 2008; Chan, 2011; Santamaria, 2006; Suresh et al., 2018; Weitzberg and Lundberg, 2013).

The human toxicity and carcinogenicity of nitrate is due to its enzymatic conversion to nitrite (NO$_2^-$) in the body, a toxic compound (Lei et al., 2018; Suresh et al., 2018; Weitzberg and Lundberg, 2013). However, recent researches have also suggested useful or not damage effects related to the intake of nitrate (Habermeyer et al., 2015; Jonvik et al., 2016). Despite this, due to the risk of excessive intake of nitrate/nitrite, the Scientific Committee on Food and the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives have established as Acceptable Daily Intake (ADI) for nitrate of 222 mg nitrate/day for a 60 kg adult, equivalent to 3.7 mg per kg body weight (World Healthy Organization, 2003). Additionally, permissible limits have been set for some leafy vegetables for European countries (European Commission, 2011).

Nitrate is widely used in greenhouses for vegetable production, especially in hydroponic growing system
(Cardenas-Navarro et al., 1999; Lei et al., 2018). However, the use and the management of nitrogen fertilizers in conventional growing system in order to improving the crop productivity also contributes to nitrate accumulation in plants (Chan, 2011; Haftbaradaran et al., 2018; Iammarino et al., 2013). Additionally, nitrate accumulation by plants is also largely influenced by biological, edaphoclimatic, and agricultural factors (such as plant cultivar, variety, light intensity, soil, temperature, and nutritional composition), as well as by storage and/or processing methods (such as storage temperature) (Alexander et al., 2008; Bian et al., 2018; Bóbics et al., 2016; Cardenas-Navarro et al., 1999; Chan, 2011; Ekart et al., 2013; Suresh et al., 2018).

Investigations carried out with vegetables has shown low levels of nitrite, but high nitrate concentrations, especially in leafy vegetables, such as Iranian vegetables (lettuce, basil, parsley, coriander, cress, dill, fenugreek, leek, mint, tarragon, spinach, and others) (Bahadoran et al., 2016), Mediterranean fresh salad vegetables (head cabbage, celly leaves, coriander, dill, chards, purslane, parsley, ruoca, and spinach (Kyriacou et al., 2019), Turkey lettuce (Aydinsakir et al., 2019), and Cyprus lettuce, spinach, ruoca, purslane, and beet leaves (Stavroulakis et al., 2018). In many cases the levels found were above the recommended limits, reinforcing the importance of monitoring nitrate levels in leafy vegetables to protect consumer health.

In this context, the aim of this study was to investigate along four weeks the nitrate and nitrite levels in commercial samples of lettuce (Lactuca sativa L.), watercress (Nasturtium officinale), spinach (Spinacea oleracea L.), and rocket (Eruca sativa Mill) cultivated in hydroponic, organic, and conventional systems.

**MATERIALS AND METHODS**

**Reagents and solutions**

All standard solutions were prepared using analytical grade reagents and deionized water (Milli-Q, Millipore, Bedford, MA, USA). Sodium nitrate, sodium nitrite, potassium thiocyanate (Internal Standard – I.S.), perchloric acid (70%), β-alanine were purchased from Sigma-Aldrich (St. Louis, CO, USA). Sodium hydroxide was purchased from Synth (Diadema, SP, Brazil). Standard solutions of the analytes and the I.S. (1000 mg L\(^{-1}\)) and stock solutions of background electrolyte (BGE) components (100 mmol L\(^{-1}\)) were prepared and stored at 4 ± 2°C until analysis when they were diluted to obtain the working concentration.

**Samples and sample preparation**

Samples of lettuce (Lactuca sativa L.), watercress (Nasturtium officinale), spinach (Spinacea oleracea L.), and rocket (Eruca sativa Mill) were prepared and stored at 4 ± 2°C until analysis when they were diluted to obtain the working concentration.

**RESULTS AND DISCUSSION**

In all leafy vegetable investigated, the nitrite concentration was below the limit of detection of the method (0.15 mg L\(^{-1}\)), while nitrate was quantified in all samples (Table 1). In a
Table 1: Nitrate content (mg kg⁻¹ in fresh weight) in lettuce, watercress, spinach and rocket grown in hydroponic, organic and conventional systems obtained from four collections

<table>
<thead>
<tr>
<th>Crop species</th>
<th>Growing system</th>
<th>Collection period (week)</th>
<th>Nitrate</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>Hydroponic</td>
<td>1</td>
<td>&lt;LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&lt;LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>963.13±40.30⁷,A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>505.25±5.87⁷,A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1</td>
<td>939.47±1.45⁰,B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1230.57±28.65⁴,A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>137.31±4.69⁴,C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1089.33±39.7⁰,A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>1</td>
<td>1513.55±74.66⁴,A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1227.22±5.12⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>291.12±5.66⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>239.26±5.83⁴-B</td>
<td></td>
</tr>
<tr>
<td>Watercress</td>
<td>Hydroponic</td>
<td>1</td>
<td>3732.34±8.04⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6036.63±137.26⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5598.38±109.69⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1</td>
<td>3629.11±115.48⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>895.25±38.87⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>887.18±39.62⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>1</td>
<td>1252.91±25.10⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1858.03±72.89⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1613.72±58.46⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1457.79±23.67⁴-B</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>Hydroponic</td>
<td>1</td>
<td>3368.29±88.45⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1298.56±50.57⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>766.71±28.13⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1</td>
<td>2892.77±125.92⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>589.28±18.88⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>277.44±12.63⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>1</td>
<td>4014.18±10.71⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3580.84±57.84⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1142.48±39.48⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3071.06±137.54⁴-C</td>
<td></td>
</tr>
<tr>
<td>Rocket</td>
<td>Hydroponic</td>
<td>1</td>
<td>7873.00±236.94⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5571.32±173.37⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>4127.43±124.46⁴-B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>1</td>
<td>6391.49±243.59⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5669.52±134.55⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5068.14±66.84⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional</td>
<td>1</td>
<td>6424.34±255.15⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6099.26±200.41⁴-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5360.05±205.78⁴-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5848.51±164.07⁴-C</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean±standard deviation. ^-^Different lowercase letters in the same growing system indicate significant differences between the means (Tukey, P<0.05). ^-^Different uppercase letters in the same collection period indicate significant differences between means (Tukey, P<0.05). LOD: Limit of detection. LOD nitrite: 0.15 mg L⁻¹

general way, it is possible to observe that nitrate presented more expressive values in the hydroponic system for lettuce, watercress and rocket. However, for spinach the highest values were found in the conventional system.

The concentrations of nitrate in the hydroponic lettuce samples ranged from 505.25 to 1531.26 mg kg⁻¹FW, for samples from the organic system from 137.31 to 1230.57 mg kg⁻¹FW and for samples produced under conventional system from...
239.26 to 1513.55 mg kg\(^{-1}\) FW (Table 1). These values are within the legal limits recommended by European Union regulations, which determines a maximum content of 4000 to 4500 mg kg\(^{-1}\) FW for lettuces harvested in the autumn/ winter and 2500 to 3500 mg kg\(^{-1}\) FW for those harvested between spring/summer period (European Commission, 2011).

Favaro-Trindade et al. (2007) found values of nitrate higher than the present study and also observed that the nitrate content in organic lettuce (565.4 mg kg\(^{-1}\) FW) was significantly lower than those determined in the conventional (2782.1 mg kg\(^{-1}\) FW) and hydroponic (3093.4 mg kg\(^{-1}\) FW) systems. Lima et al. (2008) also found higher values of nitrate for hydroponic lettuces, followed by the conventional system and, finally, the organic system. In contrast, the values found by these authors were lower than the present study, since those samples were collected during the summer (high luminosity period), where nitrate accumulation is generally lower.

In relation to watercress, the samples produced by the hydroponic system showed the highest concentrations of nitrate (3629.11 to 6036.63 mg kg\(^{-1}\) FW), followed by organic (887.18 to 2407.64 mg kg\(^{-1}\) FW) and conventional (1252.91 to 1858.03 mg kg\(^{-1}\) FW) systems (Table 1). There is no legislation for nitrate content for watercress, however, other authors have also found high values of nitrate in this vegetable cultivated in hydroponic (2009 to 6100 mg kg\(^{-1}\) FW), organic (3340 to 5926 mg kg\(^{-1}\) FW) and conventional (296 to 2388 mg kg\(^{-1}\) FW) systems (Guadagnin et al., 2005).

The nitrate concentrations in the spinach samples evaluated varied from 766.71 to 3368.29 mg kg\(^{-1}\) FW for hydroponic system, from 277.44 to 2892.77 mg kg\(^{-1}\) FW for the organic system and from 1142.48 to 4014.18 mg kg\(^{-1}\) FW in conventional system (Table 1). Samples of the conventional and hydroponic spinach plants analyzed were above the stipulated limit by European Union (3000 mg kg\(^{-1}\) FW), whereas the organic spinach plants presented contents according to the regulation. A study by Kreutz et al. (2012) reported values from 1441.5 to 2141.2 mg kg\(^{-1}\) FW for spinach grown in the conventional system and from 1150.3 to 1771.3 mg kg\(^{-1}\) FW for organic spinach samples. Nitrate levels lower than the present study were also found by Stertz et al. (2004), which reported values of up to 311.01 mg kg\(^{-1}\) FW for spinach in the conventional system and up to 614.96 mg kg\(^{-1}\) FW for organic spinach. As described for lettuce, these lower levels of nitrate for spinach described by other authors may be related to the fact that the samples analyzed by them were collected in the summer.

Lastly, nitrate levels in the rocket samples ranged from 4127.43 to 7873.00 mg kg\(^{-1}\) FW for the hydroponic system, from 6391.49 to 4546.59 mg kg\(^{-1}\) FW in the organic system and from 6424.34 to 5360.05 mg kg\(^{-1}\) FW in the conventional system (Table 1). For rocket, the stipulated limit by European Union is 7000 mg kg\(^{-1}\) FW for plants cultivated in the winter. In this way, only one sample of hydroponic rocket do not comply with this regulation. High nitrate values in hydroponically cultivated rocket (6461 to 9760 mg kg\(^{-1}\) FW) were also reported by Guadagnin et al. (2005). As in the present study, nitrate levels in rocket cultivated in the hydroponic system were followed by the samples grown in conventional and organic systems.

The nitrate contents found for the four leafy vegetables evaluated are in accordance with the classification described by Colla et al. (2018), which describes the nitrate content in lettuce as medium, high in spinach, very high in watercress and extremely high in rocket. The differences in nitrate levels found among the different evaluated vegetables may be related to the different absorption and transportation degrees in each plant (Guadagnin et al., 2005). In addition, the significant variations between the collections and between the growing systems for the same vegetable confirm that different factors can affect the concentration of nitrate in the plants, since the leafy vegetables have different behaviors compared to the factors exposed to them, such as pH, incidence of sunlight, type of fertilization, harvest time, soil composition, among others (Correia et al., 2010).

Regarding the growing system, the highest concentrations of nitrate observed for most samples of the hydroponic system is related to its availability in the system, since the nitrate is totally free, allowing the complete absorption of the nutrient solution and, consequently, greater accumulation in hydroponic vegetables compared to those grown in other systems (Colla et al., 2018; Stertz et al., 2004). The vegetables grown in the conventional growing system also tend to present higher values of nitrate in relation to the organic system, because it makes use of chemical fertilizers of medium and high solubility and concentration (Stertz et al., 2004). The organic system usually presents lower values of nitrate due to the use of organic fertilizers that contain the nutrients in the form of nitrogen salts and organic compounds, thus contributing to a lower accumulation of nitrate (Colla et al., 2018; Guadagnin et al., 2005).

**CONCLUSIONS**

This study demonstrated that the lettuce can be highlighted as the vegetable with the lowest content of nitrate, while the rocket showed the highest concentration of this compound. The higher concentrations of nitrate
were observed for the leafy vegetables growing in the hydroponic system when compared to those growing in conventional and organic systems. In addition, the nitrate content presented a great variation among the r collections performed, suggesting the influence of edaphoclimatic and agricultural factors. Despite the great variability, the nitrate levels found for most samples are in accordance with international requirements.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors also wish to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC) for the fellowships and financial support.

Author’s contributions

In this research, all authors contributed effectively. Ana Luiza do Nascimento, Fabiana Della Betta, and Luciano Valdemiro Gonzaga designed and achieved experiments, and analysed the data; Ana Luiza do Nascimento, Fabiana Della Betta, Mayara Schulz, Fabiôla Carina Biluca, Siluana Katia Tischer Seraglio performed data interpretation and wrote the paper; Luciano Valdemiro Gonzaga, Ana Carolina Oliveira Costa, and Roseane Fett supervised the project and revised the manuscript.

REFERENCES


Lima, J. D., S. Moraes, S. Helena, M. Gorla, F. N. Ibrahim and A. Carlos. 2008. Acúmulo de compostos nitrogenados e atividade...


