Determination of total oxidized nitrogen in organic and conventional herbal infusions using high-resolution continuum source graphite furnace molecular absorption spectrometry

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ABSTRACT

Excess nitrogen is stored in plants as oxidized nitrogen species, mainly nitrate and nitrite molecules, whose determination is important, especially due to their toxicity. This study evaluated the potential of high-resolution continuum source graphite furnace molecular absorption spectrometry to directly determine the total oxidized nitrogen species in diverse organic and conventional herbal infusions. Several organic carbon interferences were found using high-resolution continuum source graphite furnace molecular absorption spectrometry. This was mainly due to the low pyrolysis temperature which was employed, because of that standard addition calibration was necessary to carry out a proper analysis. The limits of detection and quantification were 0.3 and 1.0 mg L^{-1}, respectively. The concentration of total oxidized nitrogen in the infusions was determined at concentrations from limit of detection up to 56 mg L^{-1}. Overall, the influence of agricultural management on oxidized nitrogen concentration in the samples under analysis could be observed. Conventional samples had a higher content than organic ones, possibly due to common fertilization practices in conventional agriculture.

1. Introduction

Herbs have been traditionally used in folk medicine because they play an important role in the population's overall health and have low-level side effects [1]. Many herbs, such as chamomile and mint, have been exploited for their medicinal properties since they are considered one of the oldest ways of dealing with diseases [2]. This therapeutic activity has been commonly associated with compounds, such as alkaloids, tannins, oils, and vitamins found in these herbs [1]. In most herbs used for therapeutic purposes, compounds are administered through the preparation of infusions in hot water; however, in this process, not only biologically active compounds but also organic and inorganic contaminants may become available [2]. Nitrate, for example, is a common contaminant found in foods of plant origin, especially as a result of uncontrolled agricultural practices. In the human body, it has been known for its ability to reduce in vivo to nitrite, which is capable of causing the irreversible conversion of hemoglobin into methaemoglobin in the bloodstream and, thus, affect oxygen transport [3]. Additionally, nitrite, as a nitrate reduction product, can react with amines and amides found in the body to form potentially carcinogenic compounds, which are capable of increasing the incidence of gastric,

bladder, and esophageal cancers [4, 5].

Some authors have already performed nitrate determination in several kinds of herbs. Kumar et al. [6] determined nitrate in black tea and kombucha infusions and found concentrations from 0.18 to 0.34 mg g^{-1}. Özcan and Akbulut [7] determined nitrate in different herbs, such as mint, fennel, and chamomile, but only detected it in fennel samples at 38.1 mg g^{-1}. Morita and Tuji [8] evaluated the distribution of nitrate uptake in *Camellia sinensis* and observed concentrations that ranged from about 60 mg kg^{-1} in leaves to 250 mg kg^{-1} in roots.

The use of nitrogen fertilizers seems to be a determining factor in nitrate contamination in food. It has already been shown by some authors who have compiled data from the literature and have stated that products grown with organic practices have lower levels of nitrate than products grown with conventional practices [9, 10]. Similar results were found by Koh et al. [11] who performed a direct comparison among different varieties of spinach cultivated under conventional and organic regimes. In the study, only one out of 27 varieties did not have any significant difference in nitrate concentration among cultures.

The determination of nitrate and/or nitrite can be carried out using diverse analytical techniques, such as chromatographic methods, ionic

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chromatography [12–14], high-performance liquid chromatography [15], gas chromatography–mass spectrometry [16], voltammetric methods [17], and spectrometric methods [18]. Spectrometric methods can be carried out, directly and indirectly, using derivatization processes that include the reduction of nitrate to nitrite and the formation of compounds with absorption in the visible [13].

Atomic absorption spectrometry was not commonly used for determining elements, such as sulfur and nitrogen since resonance lines of these elements are below 180 nm [19]. However, with the evolution of instrumentation, new potentialities were achieved, particularly in the case of non-metal determination. High-resolution continuum source molecular absorption spectrometry (HR-CS MAS) proposes molecular determination of species, such as NO, CS, and PO that have just lately been determined using conventional atomic absorption equipment [20].

Nitrogen determination based on the NO molecule using HR-CS MAS was highlighted among spectrometric methods due to the study carried out by Huang et al. [21], who used a graphite furnace as the atomizer in 2010. New studies, which used the high-resolution molecular absorption spectrometry continuous source in graphite furnace [22, 23], flame [24] and quartz cell technique [25] followed. All methods proposed so far have determined nitrogen species in solutions at low carbon concentrations. However, this characteristic is particularly important, especially when a graphite furnace is used as the atomizer, due to the low pyrolysis temperatures that are required as a function of the volatility of the analyte.

The objective of this study was the optimization of the high-resolution continuum source molecular absorption in graphite furnace method for direct determination of total oxidized nitrogen (nitrate + nitrite) in organic and conventional herbal infusions.

2. Experimental

2.1. Instrumentation

Measurements were carried out using a ContrAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with a transversely heated graphite furnace. A xenon short-arc lamp with a nominal power of 300 W, operating in hot-spot mode, was the light source. The double monochromator (prism and echelle grating) combined with a CCD array detector with 588 pixels ensured the unique resolution with a spectral bandwidth below 2 pm/pixel. Peak area absorbance values were measured. The primary analytical line, 215.3714 nm, for NO was used for the measurement. Pyrolytically coated graphite tubes (Analytik Jena), with preinstalled PIN platform and integrated with transverse heating, were used. Argon (99.999%, White Martins, Brazil) was used as the purge gas; the internal gas flow in the graphite tube was interrupted during the atomization step.

2.2. Reagents

The 1000 mg L^{-1} N-NO standard solution was prepared by dissolving 0.303 g sodium nitrate (Merck, Germany) in 50 mL water. The 10 g L^{-1} Ca^{2+} modifier solution was prepared by dissolving 0.692 g CaCl$_2$ (Vetec, Brazil) in 25 mL water. The 10 g L^{-1} Ba^{2+} modifier solution was prepared by dissolving 0.444 g BaCl$_2 \cdot$ 2H$_2$O (Dinâmica, Brazil) in 25 mL water. The 10 g L^{-1} Sr^{2+} modifier solution was prepared by dissolving 0.760 g SrCl$_2 \cdot$ 6H$_2$O (Synth, Brazil) in 25 mL water. The stock solution of the organic carbon was prepared by dissolving 2.5 g glucose (Synth, Brazil) in 50 mL volumetric flasks. All solutions were prepared with ultrapure water.

2.3. Sample

All samples under analysis, i.e., organically and conventionally grown herbs, were bought at the local market. Herbs used for preparing infusions in the study of agricultural management were: 4 green teas (3 organic ones and 1 conventional one); 2 conventional black teas; 1 conventional white tea; 4 lemongrass teas (2 organic ones and 2 conventional ones); 3 fennel teas (1 organic one and 2 conventional ones); 2 chamomile teas (1 organic one and 1 conventional one); 3 lemon balm teas (1 organic one and 2 conventional ones); and 6 mint teas (1 organic one and 5 conventional ones).

2.3.1. Herbal infusion preparation

Herbal infusions were prepared by adding 30 mL ultrapure water, heated to 90 °C, to a sachet with the plant material. Then, they rested for 10 min to complete the infusion. Over time, the sample was homogenized as the sachet simulated the preparation of a domestic infusion.

2.4. Matrix effect determination

Matrix effects were investigated in agreement with Economou [28]. Standard addition calibration solutions were prepared in herbal infusions and compared with standard calibration solutions in water. The ratios for Sm/Ss for the slope in the sample, Sm, over the slope in ultrapure water, Ss, at different herbal infusions for the nitrate, were calculated and the extent of the effects due to matrix components was rated according to the % signal enhancement or suppression, matrix effect (%ME), which was calculated as:

$$\%ME = 100 \times (1 - (Sm/Ss))$$

Interference was classified into three levels: low effect (ranging from −20 to +20%); medium effect (from −50 to +50%); and high effect (above these values).

Organic carbon solutions used in the study of organic carbon were prepared from a previously prepared stock at 5% (wv^{-1}) C concentration in the organic form with glucose (Synth, Brazil).

2.5. Organic carbon determination

Organic carbon determination was performed using the procedure described by Gelman et al. [29], with some changes. One milliliter for sample was treated with 5 mL 0.8 mol L^{-1} potassium dichromate (Synth, Brazil) solution followed by the addition of 10 mL concentrated sulfuric acid (Vetec, Brazil). The mixture was gently swirled and left at room temperature for 16–18 h and then 100 mL ultrapure water was added to the mixture. Excess dichromate was estimated with standard ferrous ammonium sulfate (Merck, Germany) of 0.4 mol L^{-1}, the indicator was 1% ferroin (Synth, Brazil). The blank titration of the acid dichromate with ferrous ammonium sulfate solution was performed without any sample addition. The organic carbon content in the sample was calculated as:

$$\text{Organic carbon (\%)} = ((AT^* A/S)^* 0.006)/v) * 100$$

where A is the volume of the potassium dichromate solution added to the sample, T is the volume of ferrous solution used in the blank titration, S is the volume of ferrous solution used in the sample titration, v is the volume of sample and 0.006 is the weight of oxidized carbon meq per 1 mL potassium dichromate 0.48 (in g).

2.6. Pyrolysis time optimization

Pyrolysis time was optimized by comparing calibration curves in ultrapure water and infusions of *Camellia sinensis* (black tea). The matrix effect was determined as described in item 2.4 at each time (20, 40, and 60 s) under evaluation.