

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

## Current Research in Food Science

journal homepage: [www.editorialmanager.com/crfs/](http://www.editorialmanager.com/crfs/)

## Research Paper

## Development of microwave assisted-UV digestion using diluted reagents for the determination of total nitrogen in cereals by ion chromatography

Lori Rastogi<sup>a</sup>, Durga Prasad Ankam<sup>a</sup>, S. Yadlapalli<sup>c</sup>, K. Dash<sup>a,b,\*</sup><sup>a</sup> National Centre for Compositional Characterization of Materials (NCCCM), Bhabha Atomic Research Centre, ECIL Post, Hyderabad, 500 062, India<sup>b</sup> Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai, 400 094, India<sup>c</sup> FirstSource Laboratory Solutions LLP, IDA, Nacharam, Hyderabad, 500 076, India

## ARTICLE INFO

## Keywords:

Cereal flour  
Ion chromatography  
Total nitrogen  
Kjeldhal method  
Microwave-UV  
Greenness assessment

## ABSTRACT

The objective of this work is to develop a microwave assisted-ultraviolet (MW-UV) digestion in the presence of dilute HCl and H<sub>2</sub>O<sub>2</sub> followed by ion chromatography (IC) measurements for the determination of total nitrogen in cereals. This approach (MW-UV-IC) requires lesser time and does not need environmentally hazardous materials as used in Kjeldhal method. Further, the developed method requires only microliter quantities of dilute HCl and few milliliters of H<sub>2</sub>O<sub>2</sub> for the matrix digestion and simultaneous conversion of nitrogen to its ionic species for the subsequent analysis by IC. At the optimized acid concentrations (200 μL of 0.1 mol L<sup>-1</sup> HCl) and microwave power, the nitrogen in the cereals flours is converted to nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) ions. The nitrogen species were separated using IonPac AS-20 and IonPac CS-17 columns and then quantified using suppressed conductivity detection. The method was applied to estimate the total nitrogen in flours of various cereals like; wheat (*Triticum aestivum*), rice (*Oryza sativa*), finger millet (*Eleusine coracana*), jowar (*Sorghum*) and pearl millet (*Pennisetum glaucum*). The results obtained using proposed method, were in good agreement with that of Kjeldhal method. Further, the precision of the values obtained by developed method was on par with the Kjeldhal method for all the tested flours as verified by F-test (n = 5 and 95% confidence limit). Additionally, greenness assessment tools like analytical Eco-scale and green analytical procedure index (GAPI) suggested the proposed MW-UV-IC method, for the determination of total nitrogen in cereal flours, to be excellently green and safe.

## 1. Introduction

Cereals like wheat, rice, corn, barley, rye, oat, and millet are staple foods in many regions around the world and contribute to more than half of human energy requirements (McCrackin et al., 2019). Plant proteins supply 65% of the totally ingested proteins globally, with 47% of this being from cereal grains (Labuschagne, 2018). In developing countries, plant proteins are often the only source of protein (Vendemiatti et al., 2008). Protein content is the most important specification for cereals since it is related to many processing properties, such as water absorption and gluten strength. Protein content forms the basis of texture and appearance of the cereal products, e.g., for the crispy products, low protein content is favorable while high protein content is desired for products with chewy texture. Protein content is also important to anticipate water absorption and dough development time for the processes

and products, because higher protein content usually requires more water and a longer mixing time to achieve optimum dough consistency (Wheat flour and flour tes, 2004). Hence, protein content is considered as the major criterion for deciding the cereal quality. Conventionally, total protein is determined by Kjeldhal method (Kirk, 1950), where various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulfuric acid (20 mL) in the presence of 10–15 g of solid catalyst like K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> requiring a digestion time of more than 2 h. The ammonium sulphate produced in the reaction is decomposed with an alkali (NaOH) and the liberated ammonia is absorbed in excess of standard solution of acid which is then back titrated with standard alkali. Mercuric oxide is added to increase the rate of organic breakdown during acid digestion. In order to avoid the problem of high amount of chemicals and hazardous materials required in the process, a more efficient Dumas combustion method was developed

\* Corresponding author. National Centre for Compositional Characterization of Materials (NCCCM), Bhabha Atomic Research Centre, ECIL Post, Hyderabad, 500 062, India. Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai, 400 094, India.

E-mail address: [dashcccm@rediffmail.com](mailto:dashcccm@rediffmail.com) (K. Dash).

<https://doi.org/10.1016/j.crfs.2021.06.001>

Received 23 December 2020; Received in revised form 21 May 2021; Accepted 8 June 2021

2665-9271/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

where; a sample of known mass is combusted at 800–900 °C in the presence of oxygen (Dumas, 1831). Though Dumas method does not require any toxic chemicals or catalyst and is faster compared to Kjeldhal method, the limitation is relatively high initial cost. Very recently two new methods based on siSVR-near-infrared spectroscopy (NIR) (Salgó and Gergely, 2012) and hyperspectral imaging (Caporaso et al., 2018) have been reported for the determination of protein content in wheat but these methods show low measurement precision that is higher impact of random errors which lowers the quality of measuring process (Mihaljev et al., 2015). Ion chromatography (IC) is an established technique for the determination of nitrogen species in different matrices like vaccines (Wang et al., 2016), uranium alloys (Verma et al., 2007), food (Jackson et al., 1991), water samples (De Borba et al., 2014), and sediments (Colina and Gardiner, 1999). In IC analysis, nitrogen content of samples has to be converted into ionic species; ammonium, nitrate or nitrite ions. In IC method, determination of  $\text{NH}_4^+$  ions involves separation by cation exchange chromatography where conductivity of acidic eluent is suppressed by cation self-regenerating suppressor (CSRS). In the CSRS,  $\text{H}^+$  ions of the eluent are converted to water on reacting with the  $\text{OH}^-$  generated in the suppressor. The large concentration of  $\text{Cu}^{2+}$  ( $\text{CuSO}_4$  used in Kjeldhal method) reacts with  $\text{OH}^-$  in the suppressor to form  $\text{Cu}(\text{OH})_2$  precipitate which clogs the CSRS and chokes the eluent flow. Further, the divalent  $\text{Cu}^{2+}$  cation binds strongly to the cation exchange column and hence require frequent cleanup of the column. Alternatively, total nitrogen is measured by oxidizing nitrogen to nitrate (de Medina et al., 1994) using peroxodisulfate followed by IC measurement. However, oxidation by persulfate has the disadvantage of high nitrogen blank as reported by Bronk et al. (Bronk et al., 2000) and was also observed in our laboratory. F.V.M. Pontes et al. (Pontes et al., 2009) developed ultrasound-assisted purge-and-trap system and ion chromatography for analyses of total nitrogen in geological samples. This approach is though simpler and more sensitive than the classical Kjeldahl steam distillation, it still uses 10 mL of  $\text{H}_2\text{SO}_4$  (10 mol  $\text{L}^{-1}$ ) and 10 mL of  $\text{KOH}$  (10 mol  $\text{L}^{-1}$ ). In another research article, C. Domini et al. (Domini et al., 2009) reported direct microwave/ultrasound-assisted digestion procedure for the determination of total Kjeldahl nitrogen. In this procedure, due to synergistic combination of electromagnetic (2.45 GHz) and mechanical (20 kHz) energies leads to reduction in digestion time and consumption of reagents. However, the digestion requires 10 mL of  $\text{H}_2\text{SO}_4$ , 5 mL of  $\text{H}_2\text{O}_2$  and 1.5 g potassium sulphate. MW-UV system is a unique kind of digestion systems where an electrodeless discharge lamp generates UV radiation in the presence of MW field (Pereira, Wiltsche, Knapp, Flores). Hence, this system, basically utilizes the photo-oxidative properties of UV along with microwave heating thus resulting in the most effective system for the degradation of organic compounds with minimal acid requirements. The decomposition of matrix during MW-UV is initiated by the formation of hydroxyl radicals ( $\text{OH}^\bullet$ ) from  $\text{H}_2\text{O}$ ,  $\text{H}_2\text{O}_2$  and  $\text{HNO}_3$  as organic compounds are highly susceptible to these radicals. The oxidative degradation property of MW-UV has been used for the successful mineralization of a variety of matrices (Hartwig et al., 2016, 2017; Cerveira et al., 2020).

In the present work, the effectiveness of microwave - assisted UV oxidation-based digestion of cereal flours in the presence of few microliters dilute  $\text{HCl}$ ,  $\text{H}_2\text{O}_2$  and boric acid is investigated. During this digestion, nitrogen is converted into  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  which are then quantified by ion chromatography. To the best of our knowledge, this approach has not been used for the determination of total nitrogen in cereal flours. Furthermore, hydrogen peroxide (major digesting reagent) is converted to water and oxygen,  $\text{HCl}$  is used in minimal quantity (200  $\mu\text{L}$  of 0.1 mol  $\text{L}^{-1}$  concentration) which is in concurrence with green chemistry principles. The contamination of ionic nitrogen species is the bottle-neck of nitrogen determination which may arise due to presence of trace amount of organic and inorganic nitrogen species in the lab wares and other materials used during analysis. This may lead to inaccurate estimation. So stringent cleaning of microwave vessels, UV lamps and chromatographic columns are required to minimize contamination of

ionic nitrogen species (nitrate, nitrite, and ammonium).

## 2. Material and methods

### 2.1. Reagents

Suprapur grade - hydrochloric acid ( $\text{HCl}$ , 30% w/v), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (30% w/v), sodium hydroxide, methane sulfonic acid (MSA), sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ) - 98% purity, boric acid ( $\text{H}_3\text{BO}_3$ ) and methyl red indicator were obtained from Merck (Bangluru, India). All sample and standard preparations were performed using deionized water (DI) with a minimum resistivity of 18 M $\Omega$  cm that was obtained from a Millipore water purification system (Synergy<sup>(R)</sup>, Merck-Millipore, Germany). The standards from which calibration solutions were prepared for the IC method were nitrite (Trace- CERT<sup>(R)</sup>, lot no. BCB7891V), nitrate (Trace- CERT<sup>(R)</sup>, lot no. BCBP0387V) and ammonium ion (Certipur<sup>(R)</sup> Batch no: HC 41944312). These were obtained from Sigma-Aldrich, Switzerland which were traceable to the SI unit and measured against the NIST SRM. Flours of wheat, rice, finger millet, jowar and pearl millet samples were purchased from local market.

### 2.2. Instrumentation

**MW-UV digestion system:** A Multiwave-3000 pressurized microwave digestion unit, equipped with quartz vessels (80 mL capacity) from Anton Paar GmbH (Graz, Austria), was used for digestion. A low-pressure cadmium discharge lamp (part no. 16846; Anton Paar GmbH) was used as the UV source.

**Ion-chromatography:** The analysis of nitrogen ionic species was performed in an ICS-3000 ion chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump, a detector compartment containing a chromatography oven and a conductivity detector (ICS-3000 series) with suppressors. The chromatography oven was utilized to help prevent baseline drift caused by temperature variation. The instrument control, data acquisition and processing were performed with Chromeleon<sup>(R)</sup> software (version 6.80). The detailed chromatographic conditions used for the analysis of ammonium, nitrate and nitrite ions are given Table 1.

### 2.3. Cleaning procedures

Microwave vessels are often contaminated with ionic species of nitrogen as in most of the routine samples nitric acid is used as a digesting medium in analytical laboratories. Hence cleaning of the microwave

**Table 1**  
Ion chromatography Operating Conditions of ICS-3000 for Analysis of nitrite, nitrate and ammonium ion.

Column for nitrite and nitrate analysis	Dionex IonPac AS20, (250 mm × 4 mm)
Guard column	Dionex IonPac AG20, (50 mm × 4 mm)
Detection	Suppressed conductivity; conductivity cell set at T = 35 °C
Injection loop volume	25 $\mu\text{L}$
Eluent, Flow rate	$\text{NaOH}$ (20 mM), 1 mL/min
Suppressor	ASRS-300 (4 mm), Current: 55 mA
Column for ammonium ion analysis	Dionex IonPac CS17, (250 mm × 4 mm) (50 mm × 4 mm)
Guard column	Dionex IonPac CG17, (50 mm × 4 mm)
Detection	Suppressed conductivity; conductivity cell set at T = 35 °C
Injection loop volume	25 $\mu\text{L}$
Eluent, Flow rate	Methane sulfonic acid (MSA) (7 mM), 1 mL/min
Suppressor	CSRS-300 (4 mm), Current: 18 mA

vessels, UV-lamps and teflon spacers placed inside the quartz microwave vessels is required. The vessels along with UV lamp setup were cleaned by adding 10 mL of 5% (w/v) H<sub>2</sub>O<sub>2</sub> to each vessel and subjected to microwave heating (600 W, 10 min) followed by cooling and thorough rinsing with DI water (18 MΩ cm). This ensured the digestion vessels to be free from any kind of inorganic or organic nitrogen species. Chromatographic columns were also cleaned methodically and the background conductivity of <1 μS was ensured before starting the analysis. Additionally, an anion trap column (ATC-HC, 9 X 75 mm) and cation trap column (IonPac CTC-1) from Dionex were used to strip trace contaminants from the eluent or deionized water. Further, the sample preparation in microwave vessels was not carried out inside normal fume hoods as the air is contaminated with vapours of ammonia and nitric acid. As microliter quantity of dilute hydrochloric acid is used in the described method, all operations are carried out on normal work benches not connected to exhaust system, but free from activities dealing with nitric acid and ammonia.

#### 2.4. Moisture correction

Moisture analysis of the flours was carried out using a moisture analyser (Sartorius, Model; MA 100; Germany) which was calibrated for the temperature and weights. For the moisture analysis ~ 500 mg of flour was uniformly spread on the weighing pan of the instrument, moisture content of the flour was displayed after the set temperature (80 °C for 5 h) program. The obtained moisture values were applied to correct the mass fraction of nitrogen in flour.

#### 2.5. Digestion of cereal flour

Various varieties of cereal flours were procured from local market and used as such without any treatment. Approximately 50 mg of flour was taken in quartz digestion vessel to which 200 μL of HCl (0.1 mol L<sup>-1</sup>), 2 mL of H<sub>2</sub>O<sub>2</sub> (30%, w/v), 1 mL of boric acid (0.5%, w/v) and 6.8 mL of H<sub>2</sub>O were added. UV lamp along with teflon spacer was then placed in each vessel very carefully. After sealing all the vessels, the contents were digested in the microwave oven at 70 bar reaction pressure. The microwave energy is reduced automatically when the reaction pressure in one of the vessels exceeds the limit of 70 bar. The microwave program used for digestion is: 200 W, 20 ramp, 20 min hold and 800 W, 15 ramp, 20 min hold followed by cooling for 30 min. After vessels were cooled, the digested samples were transferred in clean perfluoroalkoxy (PFA) vials. The samples were diluted 1:10 times for NH<sub>4</sub><sup>+</sup> and 1:5 times for (NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>) with DI water. The calibration standards of anions were spiked with 40 μL of HCl (0.1 mol L<sup>-1</sup>) for matrix matching of the calibrant with the diluted MW-UV digest with respect to chloride ion prior to IC measurements. MW-UV digested solutions were filtered using 0.45 μm membrane based syringe filter prior to analysis by ion chromatography. This step ensures the sample to be free from any particulate matter thus prevents blockages of plumbing lines, column frits, and other components.

#### 2.6. Determination of total organic carbon content

The total organic carbon (TOC) content of the digested flour solutions was measured using a TOC analyser (TOC-VCN 5000 A, Shimadzu, Japan). TOC was measured from a calibration graph (0–50 mg L<sup>-1</sup>) using potassium hydrogen phthalate (C<sub>8</sub>H<sub>5</sub>KO<sub>4</sub>) as a stock standard solution (1000 mg L<sup>-1</sup>) for carbon.

#### 2.7. Kjeldhal digestion

The nitrogen content determination by Kjeldhal method is a three steps procedure involving digestion, distillation and titration. For digestion, 1.0 g of sample was taken in a digestion tube and to this 5 g of (1:9) (CuSO<sub>4</sub> 5H<sub>2</sub>O:Na<sub>2</sub>SO<sub>4</sub>) and 25 mL of H<sub>2</sub>SO<sub>4</sub> were added. The flask

was kept for digestion until greenish/bluish color appears. After the digestion was complete the flask was connected to distillation unit. Then, 100 mL of NaOH (40% w/v) solution was added to digestion tube and immediately distillation process was started, ammonia liberated in the process was dissolved in boric acid (50 mL, 3% w/v) present in the collection flask. For the quantitation of nitrogen content, the excess boric acid was titrated with 0.1 N HCl solution using methyl red indicator. The titer values were noted for both sample and blank (without sample) and nitrogen content was determined using following formula:

$$\% \text{ Nitrogen content} = ((S-B) * N * F) / W$$

Where,

S: Sample titer value

B: Blank titer value

N: Normality of 0.1 N HCl

W: Sample Weight

F: Nitrogen factor (F = 1.4007)

### 3. Results and discussion

#### 3.1. Optimization of microwave assisted-UV digestion of wheat flour

UV irradiation is considered as an effective and environment friendly approach for mineralization of organic samples (Dahlén et al., 1996; Moorhead and Callaghan, 1994; Singh and Uma, 2009). It is a well-known fact that the nitrogen of an organic compound as well as ammonium ions are oxidized to nitrate and nitrites during UV irradiation in the presence of small amount of oxidants (Golimowski and Golimowska, 1996). Since, UV- irradiation eliminates the need of highly concentrated acids and external catalyst for digestion, it overcomes the drawback of Kjeldhal method, and in addition the composition of digested samples is suitable for ion chromatographic analysis. Additionally, hydrogen peroxide (major digesting reagent) is converted to water and oxygen which does not contribute to the background in the conductivity detector. However, one drawback of UV-photolysis is the long irradiation time of 1–24 h (De Borba et al., 2014). But UV-photolysis in combination with MW reduces the digestion time to half an hour for many matrices (Pereira, Wiltche, Knapp, Flores; Hartwig et al., 2017; Hartwig et al., 2016; Cerveira et al., 2020; Dash et al., 2012, 2016; Iop et al., 2017; Mesko et al., 2015). This formed the basis of using microwave assisted UV digestion of cereal flours in our experiments. Wheat flour contains highest N-content among all the cereals and hence was used to optimize the digestion parameters, using which the N-content matched with those obtained from well established Kjeldhal method. Optimization of various parameters like; concentration of H<sub>2</sub>O<sub>2</sub>, HCl and effect of pH and boric acid was carried out which is discussed stepwise in following sections. The optimized conditions were then used for the digestion of all other cereal flours.

#### 3.2. Optimization of H<sub>2</sub>O<sub>2</sub> concentration

Photo-oxidation rate of organic compounds are found to increase with the addition of oxidants (Çatalkaya et al., 2003; Fernandes et al., 2019; Miklos et al., 2019; Sharma et al., 2012; Wan et al., 2019). Digestion of flour matrix takes place due to high reactivity of hydroxyl radicals which are generated from the hydrogen peroxide when exposed to UV radiation inside the microwave vessel as follows Eqn 1:



Addition of H<sub>2</sub>O<sub>2</sub> ensures super saturation of samples with oxygen. The generated hydroxyl radicals initiate the radical chain reaction with the organic matrix. Further, H<sub>2</sub>O<sub>2</sub> decomposes to H<sub>2</sub>O and O<sub>2</sub> Eqn 2.



UV irradiation in the presence of oxygen leads to oxidation of nitrogen containing organic compounds initially to nitrates which are then slowly reduced to nitrites. As a result, total nitrogen is converted to  $\text{NO}_2^-$  and  $\text{NO}_3^-$  after digestion equation Eqn 3 (Golimowski and Golimowska, 1996).

UV (200–300 nm)



The wheat flour was digested in the presence of various concentrations (3–12% w/v) of  $\text{H}_2\text{O}_2$  (this was obtained by diluting 30% w/v solution which is commercially available) and the digested solutions were analysed by ion chromatography for assessment of total nitrogen. All the three species ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) were analysed using respective analytical columns by IC and nitrogen content of flour was calculated by adding the N of all the species. Effect of  $\text{H}_2\text{O}_2$  concentration on the digestion of wheat flour under microwave assisted UV digestion can be explained by the data tabulated in Table 2. It is evident that the recovery of total nitrogen content increased with increase in  $\text{H}_2\text{O}_2$  concentration. While at 3% (w/v) of  $\text{H}_2\text{O}_2$  the flour remained in charred form and at 6–12% (w/v), though a transparent solution was obtained, but the recoveries of total nitrogen obtained were less as compared to Kjeldhal method. It can be inferred from the data that  $\text{H}_2\text{O}_2$  alone was not able to digest the flour or convert organic - N to anions quantitatively, since the maximum recovery obtained; in terms of total nitrogen were only 12% (w/w) at highest amount of  $\text{H}_2\text{O}_2$  used, as compared to Kjeldhal method.

### 3.3. Optimization of HCl concentration and pH

The N-content in cereal flours is mostly present in protein which is a polypeptide of amino acids. Such flours are complex matrix where the proteins are present in various secondary and tertiary forms, and as observed before,  $\text{H}_2\text{O}_2$  alone does not convert organic nitrogen to nitrogen ions. It is well known that protein undergo acid hydrolysis into their constituent amino acids as shown in the following schematic:

The conventional acid hydrolysis of polypeptide requires  $\sim$  HCl (6 mol  $\text{L}^{-1}$ ) and use of such high concentration of acid renders the digest incompatible for the separation of N-anions by alkaline eluent. So, low concentration of HCl (0.5–5.0 mol  $\text{L}^{-1}$ ) and 1 mL of boric acid (0.5%, w/v) solution were included in the digestion solution in the presence of 6% (w/v)  $\text{H}_2\text{O}_2$  so as to improve the digestion efficiency. It is clear from Fig. 1 that the recoveries of total nitrogen increased with increase in acid concentration from 0.5 to 2.0 mol  $\text{L}^{-1}$  and then decreased with further increase in the acid strength but total recoveries remained higher than the digestions which were done in the absence of acid. Such higher recovery of N is possible as the hydrolysis of protein results into constituent amino acids and UV photolysis converts amino-N of amino acid to  $\text{NH}_4^+$  which is again partly photo-oxidized to  $\text{NO}_3^-$  and  $\text{NO}_2^-$  as reported by Manny et al. (Manny et al., 1971). This observation was also in concurrence with W. Dorten et al. who suggested that acidifying (pH 2.0) the mineralized samples and subsequent photo-oxidation synergistically enhance the efficacy of photo-oxidation (Dorten et al., 1984). Further,

**Table 2**  
Mineralization of wheat flour in the presence of various concentrations of  $\text{H}_2\text{O}_2$ .

S. N.	$\text{H}_2\text{O}_2$ Concentration	Total nitrogen (g/100 g) <sup>a</sup>
1	3	Incomplete digestion
2	6	0.17 ± 0.023
3	9	0.194 ± 0.018
4	12	0.245 ± 0.014

<sup>a</sup> Values are based on five independent analysis; Values mean ± SD.

investigation of the digested solution revealed the difference in the pH of the digested solutions which are tabulated in Table 3. It can be seen that the sample with highest N-recovery (2.04 g/100 g) was obtained when HCl (2.0 mol  $\text{L}^{-1}$ ) was used in the MW-UV digestion and pH after digestion was 6.46. It was observed that all the three species of nitrogen i.e. nitrate, nitrite and ammonium are formed as a result of digestion at all the pH and ammonium ion was found to be the dominant species. The representative chromatograms of these nitrogen ions obtained after MW-UV digestion is given in figure - 2. For the separation of  $\text{NH}_4^+$  by IC column (IonPacCS17) with carboxylic acid functional group is used. These carboxylate types of columns have similar selectivities for  $\text{NH}_4^+$ ,  $\text{Na}^+$  and  $\text{K}^+$  where the latter two ions are present in the flour matrix. However, as  $\text{NH}_4^+$  is the dominant species formed during the conversion of N in cereals under the described procedure, a large dilution factor of 2000 was used during the IC measurement stage. With such large dilution factor, the  $\text{NH}_4^+$  peak well separated from the  $\text{Na}^+$  and  $\text{K}^+$  peaks (Fig. 2). For the separation of anions ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ), chloride ion is the dominant matrix, which is added during the UV digestion step. After a dilution factor of 1000,  $\text{NO}_3^-$  peak is resolved from the large chloride peak, but the base line is slightly elevated in the left. This is taken care by adding proportional quantity of hydrochloric acid (0.1 mol  $\text{L}^{-1}$  HCl) to the anion calibrants. At the optimized acid concentration (2.0 mol  $\text{L}^{-1}$  HCl), the amount of  $\text{H}_2\text{O}_2$  was increased (6–12% w/v) in the hope that all  $\text{NH}_4^+$  will be further quantitatively oxidized to nitrate (due to high oxygen levels) since sufficient  $\text{O}_2$  is required to convert all N of  $\text{NH}_4^+$  to nitrate (Armstrong et al., 1966). By such quantitative conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  only anion chromatography will be required instead of both cation and anion type. However, even at higher  $\text{H}_2\text{O}_2$  concentrations,  $\text{NH}_4^+$  remained as the dominant species without any significant change in the ratio of  $\text{NH}_4^+$ :  $\text{NO}_2^-$ :  $\text{NO}_3^-$  (Table 4). Further, the pH of all the samples after digestion varied within 6.46–6.56. Malaiyandi et al. (Murugan Malaiyandi et al., 1980) has reported that the oxidation by hydroxyl radicals is most efficient in the pH range of 6–8. So, the acid hydrolysis was replaced by alkaline hydrolysis anticipating whether quantitative conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  could be achieved. The digestion of flour in the presence of NaOH,  $\text{H}_2\text{O}_2$  and boric acid led to the 100% recoveries of total nitrogen but here also  $\text{NH}_4^+$  remained as the dominant species. It is important to note that the pH of the digested solution after digestion was between (7.7–7.8) and there was increase in % of  $\text{NO}_3^-$  in the final digest. This is because in alkaline pH, conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  and  $\text{NO}_2^-$  is favored. So, it is observed that the organic nitrogen in flour is converted to  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in the pH ranging from 2.6 to 7.8. It is also inferred from the results that the pH played very important role in quantitative recovery of total nitrogen and two chromatographic conditions (cationic and anionic) were used to quantify the positively and negatively charged species of nitrogen. A pH range of 6.4–7.8 was found to be optimum whereas at lower pH recoveries were not quantitative. We did not carry out UV digestion at pH > 8, as it is reported that photolytic efficiency is reduced beyond this pH. Effect of pH on the photo-oxidation has been studied by many researchers (Henriksen, 1970; Lan et al., 2016; Ren et al., 2016).

### 3.4. Effect of boric acid on the quantitative recovery of total nitrogen

The boric acid at acidic pH converts ammonia gas to ammonium ions and borate ions are formed in the process Eqn 4:



Recovery of total nitrogen and  $\text{NH}_4^+$  ion concentration was found to decrease in the absence of boric acid because some of the nitrogen is lost as ammonia gas. The optimization studies indicated that a digestion solution constituted of (6% w/v  $\text{H}_2\text{O}_2$  + 2.0 mol  $\text{L}^{-1}$  HCl + 0.05% w/v, Boric acid) is optimum for the quantitative recovery of total nitrogen of wheat flour under microwave assisted UV irradiation. These parameters were used for the MW-UV digestion of all the cereal flours.

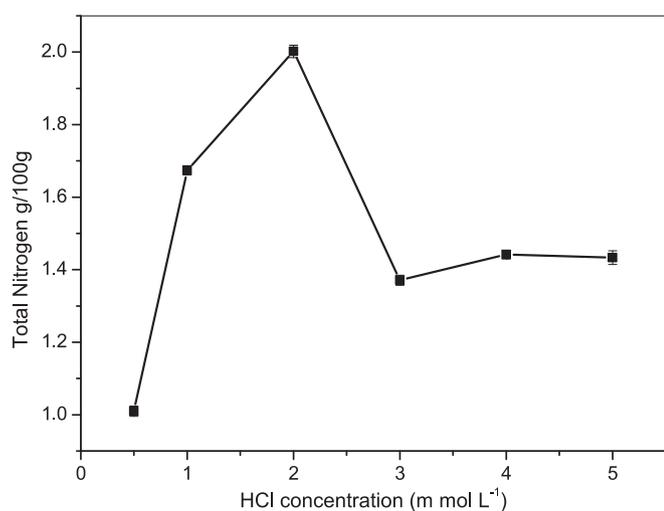
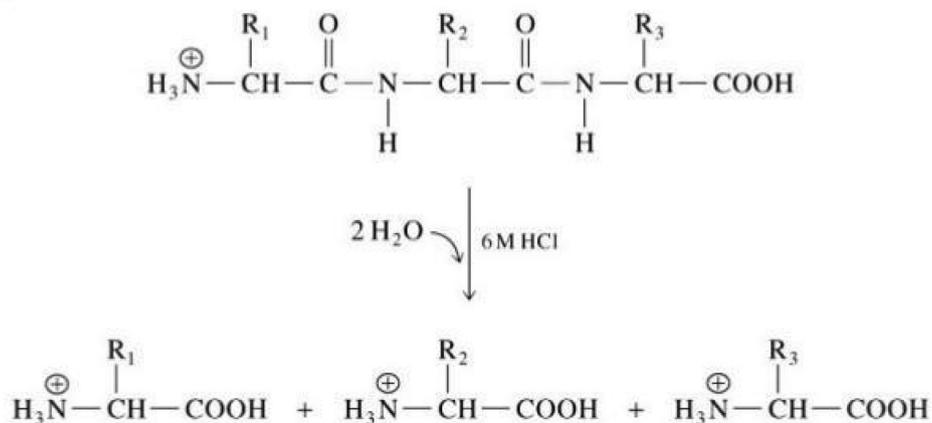


Fig. 1. Effect of acid concentration on the recovery of total nitrogen.

Table 3

Effect of HCl concentrations on total N recovery in refined wheat flour and fraction of N (g/100 g) from  $NH_4^+$ ,  $NO_2^-$  and  $NO_3^-$  respectively.

S. N.	HCl (m mol L <sup>-1</sup> )	pH of sample after digestion	Total N (g/100 g) <sup>a</sup>	$NH_4^+$ : $NO_2^-$ : $NO_3^-$
1	0.5	6.64	1.01 ± 0.014	0.74 : 0.261 : 0.006
2	1.0	6.56	1.67 ± 0.013	1.39 : 0.280 : 0.0005
3	2.0	6.46	2.04 ± 0.017	1.77 : 0.264 : 0.0008
4	3.0	3.26	1.37 ± 0.014	1.13 : 0.230 : 0.002
5	4.0	2.8	1.44 ± 0.012	1.18 : 0.256 : 0.002
6	5.0	2.73	1.43 ± 0.019	1.14 : 0.284 : 0.008

<sup>a</sup> Values are based on five independent analysis; Values mean ± SD.

The mineralization efficiency of the developed MW-UV method with optimized quantity of reagents was evaluated by determination of total

organic carbon content in the final digests. The TOC contents of the final digested solutions were found to be in the range from 0.62% to 1.2%. The lower TOC contents indicated efficient mineralization of the matrix (Pereira, Wiltische, Knapp, Flores; Cerveira et al., 2020) and its suitability for analysis by IC.

### 3.5. Determination of total nitrogen content of various cereals using the developed method

After optimizing the MW-UV-IC based method for the determination of total nitrogen in wheat flour, it was then applied to other cereals flours. The cereal flours tested were: rice, finger millet, jowar and pearl millet. The total nitrogen content using MW-UV digestion under optimized conditions and followed by IC analysis and the results are presented in Table 5. The N-contents were 1.91, 2.03, 1.32, 1.28, 1.73, 1.67 (g/100 g) for refined wheat flour, whole wheat flour, rice finger millet, jowar and pearl millet respectively. These values were in concurrence with the values obtained by Kjeldhal method. The most important feature of the proposed method is related to greenness. An amount of ~ 50 mg of the sample is digested using minimum amount of reagents with final solution concentration of  $H_2O_2$  (6% w/v) + HCl (2.0 m mol L<sup>-1</sup>) + Boric acid (0.05% w/v). The obtained digested solution is sufficient and suitable to carry out the analysis for the determination of protein content in the cereal flours. The homogeneity of sample at this level (~ 50 mg) was assured using Kurfurst equation ( $H_E = \% R. S. D. \times \sqrt{\text{mass (mgH} = \sqrt{\text{mass(mg)} \times \%R.S.D)}$ ) (Kurfürst et al., 1984), the obtained  $H_E$  value was less than 10 in all the cases (supplementary table –1). Thus, the proposed method used an amount of reagents several times lower than the classical method and assure the compatibility with detection technique as ion chromatography.

### 3.6. Analytical figures of merit and process blanks

Calibration was found to be linear from 5 - 25 mg L<sup>-1</sup> for the  $NH_4^+$ , 2.5 - 10 mg L<sup>-1</sup> for  $NO_3^-$  and  $NO_2^-$  with  $R^2 > 0.99$ . The limit of detection (LOD) of the method was calculated from the calibration graph as  $3.3 \times S_{x/y}/\text{slope}$ , where  $S_{x/y}$  corresponds to error in Y-intercept. LOD for  $NH_4^+$ ,  $NO_3^-$  and  $NO_2^-$  in terms of N is found to be 0.00011,  $5.5 \times 10^{-6}$ ,  $2.45 \times 10^{-6}$  g/100 g of flour respectively. Similarly, limit of quantification (LOQ) was calculated as  $10 \times S_{x/y}/\text{slope}$  and was found to be 0.00033,  $1.66 \times 10^{-5}$  and  $7.44 \times 10^{-6}$  g/100 g of flour respectively. The process blank values for  $NH_4^+$ ,  $NO_2^-$  and  $NO_3^-$  of the optimized digested solutions were  $2 \times 10^{-3}$ ,  $1.2 \times 10^{-4}$  and  $5.7 \times 10^{-6}$  (g/100 g) of flour which indicated that the contamination was significantly minimized for such ubiquitous analytes.

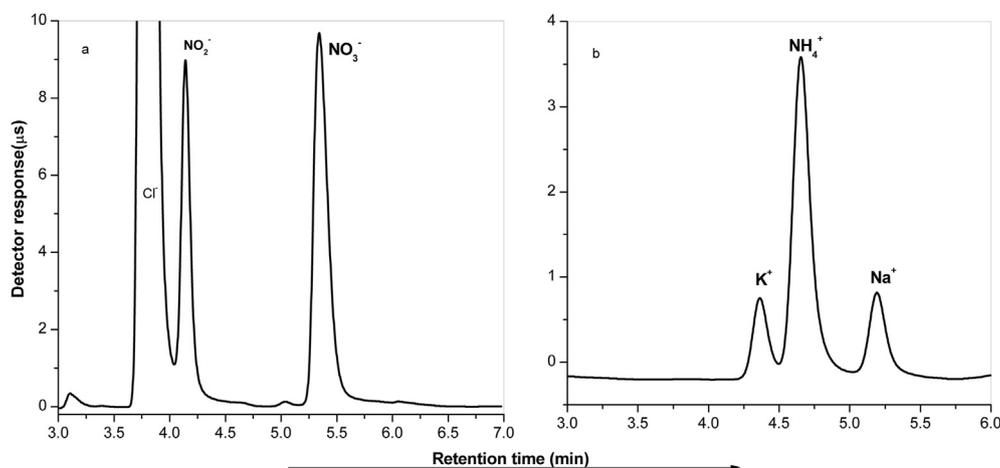


Fig. 2. The representative chromatograms of (a) nitrite, nitrate and (b) ammonium ion analysis in digested flour solution.

Table 4

Effect of H<sub>2</sub>O<sub>2</sub> on MW-UV digestion of flour in the presence of 2.0 m mol L<sup>-1</sup> HCl in terms of total nitrogen.

S. N.	H <sub>2</sub> O <sub>2</sub> (%)	pH of sample	Total Nitrogen (g/100 g) <sup>a</sup>	NH <sub>4</sub> <sup>+</sup> : NO <sub>2</sub> <sup>-</sup> : NO <sub>3</sub> <sup>-</sup>
		after digestion		
1	3	6.64	Incomplete digestion	
2	6	6.56	2.05 ± 0.015	1.79 : 0.255 : 0.007
3	9	6.46	2.08 ± 0.013	1.84 : 0.231 : 0.002
4	12	6.52	2.07 ± 0.017	1.82 : 0.250 : 0.003

<sup>a</sup> Values are based on five independent analysis; Values mean ± SD.

### 3.7. Accuracy and precision of the method

Accuracy of the method is established by spiking known concentration of standards to the digested solutions and calculating the recovery after IC analysis. The results are shown in Table 6. The recoveries of the analytes varied from 96 to 101% suggesting that the method is highly accurate for the analysis of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> in digested flour solution.

Table 5

Comparison total nitrogen content of various cereals as obtained by Kjeldhal and MW-UV-IC method.

Name of the cereal flour	Total Nitrogen (g/100 g)	
	(Mean ± S.D.) (n = 5)	
	Kjeldhal Method	Present method
Whole wheat	1.91 ± 0.005	1.92 ± 0.015
Refined wheat	2.03 ± 0.005	2.03 ± 0.017
Rice	1.32 ± 0.006	1.30 ± 0.012
Finger millet	1.28 ± 0.007	1.29 ± 0.011
Jowar	1.73 ± 0.006	1.72 ± 0.014
Pearl millet	1.69 ± 0.006	1.70 ± 0.009

Table 6

Spike and recovery results of various analytes to evaluate the accuracy and influence of sample matrix on their analysis using IC.

Analyte	Added (mg L <sup>-1</sup> )	Obtained (mg L <sup>-1</sup> )	Recovery (%) (n = 3)
NH <sub>4</sub> <sup>+</sup>	12.5	12.7	99 ± 2.5
NO <sub>3</sub> <sup>-</sup>	2.5	2.6	104 ± 3.0
NO <sub>2</sub> <sup>-</sup>	2.5	2.4	98 ± 3.0

The good recoveries also indicated that there is no significant influence of residual carbon on the determination of analytes. In absence of any certified reference material of flour in our laboratory, the accuracy of the developed method is further established by comparing the results with Kjeldhal method. The values of total nitrogen contents of all the tested cereals obtained with the developed MW-UV-IC approach was in close agreement with Kjeldhal method (Table 5). The application of *t*-test (n = 5 and 95% confidence limit) demonstrated that there is no significant difference in mean nitrogen content of five replicate measurements of cereal flours by both the methods. The precision of the developed method in terms of relative standard deviation (RSD) was found to vary between 0.53% and 0.91%. Additionally, the precision of the values obtained by developed method was on par with the Kjeldhal method for all the tested flours as verified by F-test (n = 5 and 95% confidence limit).

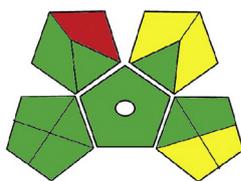
### 3.8. Assessment of greenness of the MW-UV-IC approach

The main purposes of green analytical chemistry concepts are to reduce and eliminate the use of hazardous chemicals leading to sustainable development. The greenness assessment of the developed MW-UV-IC approach for the digestion of wheat flour has been carried out using two widely accepted methods analytical Eco-scale score (Galuszka et al., 2012) and Green Analytical Procedure Index<sup>®</sup> (GAPI) tool (Wasyłka, 2018). Analytical Eco-scale score is a semi-quantitative approach where a score is calculated by subtracting penalty points (for the parameters of analytical process which are not in compliance with green chemistry concepts) from 100. Whereas, GAPI uses a five-pentagram symbol to evaluate and quantify the greenness of an analytical method with color codes green, yellow and red suggestive of low, medium and high impact on the environment. Analytical Eco-score of the proposed MW-UV-IC approach was found to be 85 (Table 7), which suggests the method to be an excellent green. The GAPI symbol of the proposed method also suggests MW-UV-IC approach to be green in nature as 10 fields are shaded green, 4 yellow and only 1 red.

**Table 7**

Greenness assessment of the proposed MW-UV-IC approach for the determination of total nitrogen in cereal flours by Analytical Eco-scale and GAPI tools.

Analytical Eco-Scale		GAPI Assessment	
Reagents	Penalty points		
H <sub>2</sub> O <sub>2</sub>	4	<b>Sample preparation</b> 1. Collection: Off-line 2. Preservation: None 3. Transport: None 4. Storage: None 5. Type of the method (direct/indirect): No 6. Scale of extraction: None 7. Solvents/Reagents: None 8. Additional treatments: None <b>Reagents and solvents</b> 9. Amounts: < 10 mL 10. Health hazard H <sub>2</sub> O <sub>2</sub> and HCl: Can cause serious or permanent injury, NFPA health hazard rating = 3 11. Safety hazard Ethanol: instability score = 1, Flammability score = 0, No special hazard <b>Instrumentation</b> 12. Energy: ≤0.1 kWh/sample 13. Occupational hazard: Hermetic sealing of the analytical process 14. Waste <10 mL 15. Waste treatment No treatment	
HCl	4		
NaOH	0		
Methane sulfonic acid	0		
<b>Instruments</b>			
Microwave digestion system	2		
Ion - chromatography	0		
Occupational hazard	0		
Waste	5		
Total penalty	15		
Eco-score	100-15 = 85		GAPI pictogram



#### 4. Conclusions

This work presents a new approach (MW-UV-IC) for the determination of total nitrogen in cereals. The method involved digestion of the flour in the presence of dilute hydrochloric acid, boric acid and hydrogen peroxide under microwave assisted UV digestion followed by ion chromatographic quantitation. The organic nitrogen is converted to NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> and the quantitative recoveries were obtained in pH range of 6.4–7.8. Total nitrogen contents obtained by MW-UV-IC approach were in close agreement with Kjeldhal method, for all the cereal flours. The proposed method requires very dilute acids for the digestion and no metallic catalyst are used. Additionally, the developed MW-UV-IC approach is in compliance with the green chemistry principles of working with safer and lesser amount of chemicals as assessed by analytical Eco-scale and GAPI directives. The N - measurement by IC is traceable to SI units as SI traceable standards of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were used for quantitation. The comparison has revealed that the developed method needs least amount of reagents, has better precision than other approaches. Among the drawbacks is the requirement of a double system ion-chromatography as both cationic and anionic species are found after the MW-UV digestion.

#### Credit author statement

**Lori Rastogi:** Ideas formulation, development of research goals along with conducting the experiments and data analysis and writing the initial draft.

**Durga Prasad Ankam:** Conducting the experiments, critical reading of the manuscript.

**S. Yadlapalli:** Has helped in the experimentation.

**K. Dash:** Oversight and leadership responsibility, execution,

including mentorship external to the core team and finalization of the manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement:

Grateful acknowledgement is made to Dr. Sanjiv Kumar Head, NCCCM/BARC for his encouragement throughout this work. We thank Palle Environmental and analytical laboratories, Hyderabad for helping with the TOC analysis.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2021.06.001>.

#### References

- Armstrong, F.A.J., Williams, P.M., Strickland, J.D.H., 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. *Nature* 211, 481–483. <https://doi.org/10.1038/211481a0>.
- Bronk, D.A., Lomas, M.W., Glibert, P.M., Schukert, K.J., Sanderson, M.P., 2000. Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. *Mar. Chem.* 69, 163–178. [https://doi.org/10.1016/S0304-4203\(99\)00103-6](https://doi.org/10.1016/S0304-4203(99)00103-6).
- Caporaso, N., Whitworth, M.B., Fisk, I.D., 2018. Protein content prediction in single wheat kernels using hyperspectral imaging. *Food Chem.* 240, 32–42. <https://doi.org/10.1016/j.foodchem.2017.07.048>.
- Çatalkaya, E.Ç., Bali, U., Şengül, F., 2003. Photochemical degradation and mineralization of 4-chlorophenol. *Environ. Sci. Pollut. Res.* 10, 113–120. <https://doi.org/10.1007/s11356-002-10135-6>.
- Cerveira, C., Hermann, P.R.S., Pereira, J.S.F., Pozebon, D., Mesko, M.F., Moraes, D.P., 2020. Evaluation of microwave-assisted ultraviolet digestion method for rice and wheat for subsequent spectrometric determination of As, Cd, Hg and Pb. *Journal of Food Composition and Analysis.* *J. Food Compos. Anal.* 92, 103585. <https://doi.org/10.1016/j.jfca.2020.103585>.
- Colina, M., Gardiner, P.H.E., 1999. Simultaneous determination of total nitrogen, phosphorus and sulphur by means of microwave digestion and ion chromatography. *J. Chromatogr. A* 847, 285–290. [https://doi.org/10.1016/S0021-9673\(99\)00024-2](https://doi.org/10.1016/S0021-9673(99)00024-2).
- Dahlén, J., Bertilsson, S., Pettersson, C., 1996. Effects of UV-A irradiation on dissolved organic matter in humic surface waters. *Environ. Int.* 22, 501–506. [https://doi.org/10.1016/0160-4120\(96\)00038-4](https://doi.org/10.1016/0160-4120(96)00038-4).
- Dash, K., Rastogi, L., Arunachalam, J., 2012. DNA quantification via traceable phosphorus measurement through microwave-assisted UV digestion-ion chromatography. *Analyst* 137, 668–674. <https://doi.org/10.1039/C2AN15883C>.
- Dash, K., Rastogi, L., Thangavel, S., Venkateswarulu, G., 2016. Traceable quantitation of cyanocobalamin (vitamin B12) via measurement of cobalt and phosphorus: a comparative assessment using inductively coupled plasma atomic emission spectrometry (ICP-AES) and ion chromatography (IC). *RSC Adv.* 6, 111090–111098. <https://doi.org/10.1039/C6RA16637G>.
- De Borja, B.M., Jack, R.F., Rohrer, J.S., Wirt, J., Wang, D., 2014. Simultaneous determination of total nitrogen and total phosphorus in environmental waters using alkaline persulfate digestion and ion chromatography. *J. Chromatogr. A* 1369, 131–137. <https://doi.org/10.1016/j.chroma.2014.10.027>.
- de Medina, H.L., de Vargas, M.C., Marin, J., Pirela, D., 1994. Determination of total nitrogen in water samples by means of high-pressure bombs and ion chromatography. *J. Chromatogr. A* 671, 287–293. [https://doi.org/10.1016/0021-9673\(94\)80252-1](https://doi.org/10.1016/0021-9673(94)80252-1).
- Domini, C., Vidal, L., Cravotto, G., Canals, A., 2009. A simultaneous, direct microwave/ultrasound-assisted digestion procedure for the determination of total Kjeldahl nitrogen. *Ultraso. sonochem.* 16, 564–569. <https://doi.org/10.1016/j.ultrasch.2008.12.006>.
- Dorten, W., Valenta, P., Nürnberg, H.W., 1984. A new photodigestion device to decompose organic matter in water. *Fres.' Zeits. für analyt. Chem.* 317, 264–272. <https://doi.org/10.1007/BF00494476>.
- Dumas, J.B.A., 1831. *Procédes de l'Analyse Organique.* *Ann. Chem. Phys.* 247, 198–213.
- Fernandes, A., Gagol, M., Makoš, P., Khan, J.A., Boczkaj, G., 2019. Integrated photocatalytic advanced oxidation system (TiO<sub>2</sub>/UV/O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>) for degradation of volatile organic compounds. *Separ. Purif. Technol.* 224, 1–14. <https://doi.org/10.1016/j.seppur.2019.05.012>.
- Galuszka, A., Konieczka, P., Migaszewski, Z.M., Namiesnik, J., 2012. Analytical Eco-Scale for assessing the greenness of analytical procedures. *Trends Anal. Chem.* 37, 61–72. <https://doi.org/10.1016/j.trac.2012.03.013>.

- Golimowski, J., Golimowska, K., 1996. UV-photooxidation as pretreatment step in inorganic analysis of environmental samples. *Anal. Chim. Acta* 325, 111–133. [https://doi.org/10.1016/0003-2670\(96\)00034-7](https://doi.org/10.1016/0003-2670(96)00034-7).
- Hartwig, C.A., Pereira, R.M., Rondan, F.S., Cruz, S.M., Duarte, F.A., Flores, E.M.M., Mesko, M.F., 2016. The synergic effect of microwave and ultraviolet radiation for chocolate digestion and further determination of As, Cd, Ni and Pb by ICP-MS. *J. Anal. At. Spectrom.* 31, 523–530. <https://doi.org/10.1039/c5ja00388a>.
- Hartwig, C.A., Pereira, R.M., Novo, D.L.R., Oliveira, D.T.T., Mesko, M.F., 2017. Green and efficient sample preparation method for the determination of catalyst residues in margarine by ICP-MS. *Talanta* 174, 394–400. <https://doi.org/10.1016/j.talanta.2017.06.037>.
- Henriksen, A., 1970. Determination of total nitrogen, phosphorus and iron in fresh water by photo-oxidation with ultraviolet radiation. *Analyst* 95, 601–608. <https://doi.org/10.1039/AN9709500601>.
- Iop, G.D., Krzyżaniak, S.R., Silva, J.S., Flores, E.M.M., Costa, A.B., Mello, P.A., 2017. Feasibility of microwave-assisted ultraviolet digestion of polymeric waste electrical and electronic equipment for the determination of bromine and metals (Cd, Cr, Hg, Pb and Sb) by ICP-MS. *J. Anal. Atomic Spectrom.* 32, 1789–1797. <https://doi.org/10.1039/C7JA00123A>.
- Jackson, P.E., Krol, J., Heckenberg, A.L., Mientijes, M., Staal, W., 1991. Determination of total nitrogen in food, environmental and other samples by ion chromatography after Kjeldahl digestion. *J. Chromatogr. A* 546, 405–410. [https://doi.org/10.1016/S0021-9673\(01\)93039-0](https://doi.org/10.1016/S0021-9673(01)93039-0).
- Kirk, P.L., 1950. Kjeldahl method for total nitrogen. *Anal. Chem.* 22, 354–358. <https://doi.org/10.1021/ac60038a038>.
- Kurfürst, U., Grobecker, K.H., Stoepler, M., 1984. Homogeneity studies in biological reference and control materials with solid sampling and direkt Zeeman-AAS. In: Schramel, P., Bratter, P. (Eds.), *Trace Elem Anal Chem Med and Biol*, vol. 3, pp. 591–601.
- Labuschagne, M.T., 2018. A review of cereal grain proteomics and its potential for sorghum improvement. *J. Cereal. Sci.* 84, 151–158. <https://doi.org/10.1016/j.jcs.2018.10.010>.
- Lan, Q., Cao, M., Ye, Z., Zhu, J., Chen, M., Chen, X., Liu, C., 2016. Effect of oxalate and pH on photodegradation of pentachlorophenol in heterogeneous irradiated maghemite System. *J. Photochem. Photobiol. Chem.* 328, 198–206. <https://doi.org/10.1016/j.jphotochem.2016.06.001>.
- Manny, B.A., Miller, M.C., Wetzal, R.G., 1971. Ultraviolet combustion of dissolved organic nitrogen compounds in lake waters. *Limnol. Oceanogr.* 16, 71–85. <https://doi.org/10.4319/lo.1971.16.1.0071>.
- McCrackin, S.D., Soomal, S.K., Patel, P., Itier, R.J., 2019. Spontaneous eye-movements in neutral and emotional gaze-cuing: an eye-tracking investigation. *Heliyon* 5, e01583. <https://doi.org/10.1016/j.heliyon.2019.e01583>.
- Mesko, M.F., Piccoloto, R.S., Ferreira, L.R., Costa, V.C., Pereira, C.M.P., Colepicolo, P., Muller, E.I., Flores, E.M.M., 2015. Ultraviolet radiation combined with microwave-assisted wet digestion of Antarctic seaweeds for further determination of toxic elements by ICP-MS. *J. Anal. Atomic Spectrom.* 30, 260–266. <https://doi.org/10.1039/C4JA00264D>.
- Mihaljević, Ž., Jakšić, S., B Prica, N., N Ćupić, Ž., Baloš, M., 2015. Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products. *J. Agroalim. Pro. Technol.* 21, 365–370.
- Miklos, D.B., Wang, W.L., Linden, K.G., Drewes, J.E., Hübner, U., 2019. Comparison of UV-AOPs (UV/H<sub>2</sub>O<sub>2</sub>, UV/PDS and UV/Chlorine) for TOC removal from municipal wastewater effluent and optical surrogate model evaluation. *Chem. Eng. J.* 362, 537–547. <https://doi.org/10.1016/j.cej.2019.01.041>.
- Moorhead, D.L., Callaghan, T., 1994. Effects of increasing ultraviolet B radiation on decomposition and soil organic matter dynamics: a synthesis and modelling study. *Biol. Fertil. Soils* 18, 19–26. <https://doi.org/10.1007/bf00336439>.
- Murugan Malaiyandi, M., Sadar, H., Lee, P., O'Grady, R., 1980. Removal of organics in water using hydrogen peroxide in presence of ultraviolet light. *Water Res.* 14, 1131–1135. [https://doi.org/10.1016/0043-1354\(80\)90164-5](https://doi.org/10.1016/0043-1354(80)90164-5).
- Pereira, J.S.F., Wiltsche, H.W., Knapp, G., Microwave-assisted ultraviolet digestion, chapter 7, in *Microwave-assisted Sample Preparation for Trace Element Determination*. first ed., Flores, E.M.M., (Editor) Elsevier; ISBN-13 978-0444594204.
- Pontes, F.V.M., Carneiro, M.C., Vaitsman, D.S., da Rocha, G.P., da Silva, L.L.D., Neto, A.A., Monteiro, M.I.C., 2009. A simplified version of the total kjeldahl nitrogen method using an ammonia extraction ultrasound-assisted purge-and-trap system and ion chromatography for analyses of geological samples. *Anal. Chim. Acta* 632, 284–288. <https://doi.org/10.1016/j.aca.2008.11.011>.
- Ren, D., Huang, B., Bi, T., Xiong, D., Pan, X., 2016. Effects of pH and dissolved oxygen on the photodegradation of 17 $\alpha$ -ethynylestradiol in dissolved humic acid solution. *Environ. Sci. Proces. Imp.* 18, 78–86. <https://doi.org/10.1039/C5EM00502G>.
- Salgó, A., Gergely, S., 2012. Analysis of wheat grain development using NIR spectroscopy. *J. Cereal. Sci.* 56, 31–38. <https://doi.org/10.1016/j.jcs.2012.04.011>.
- Sharma, S., Mukhopadhyay, M., Murthy, Z.V.P., 2012. Rate parameter estimation for 4-chlorophenol degradation by UV and organic oxidants. *J. Ind. Eng. Chem.* 18, 249–254. <https://doi.org/10.1016/j.jiec.2011.11.033>.
- Singh, J., Uma, S., 2009. Efficient photocatalytic degradation of organic compounds by ilmenite AgSbO<sub>3</sub> under visible and UV light irradiation. *J. Phys. Chem. C* 113, 12483–12488. <https://doi.org/10.1021/jp901729v>.
- Vendemiatti, A., Rodrigues Ferreira, R., Humberto Gomes, L., Oliveira Medici, L., Antunes Azevedo, R., 2008. Nutritional quality of sorghum seeds: storage proteins and amino acids. *Food Biotechnol.* 22, 377–397. <https://doi.org/10.1080/08905430802463487>.
- Verma, P., Rastogi, R.K., Ramakumar, K.L., 2007. Determination of trace amounts of nitrogen in uranium based samples by ion chromatography (IC) without Kjeldahl distillation. *Anal. Chim. Acta* 596, 281–284. <https://doi.org/10.1016/j.aca.2007.06.019>.
- Wan, Y., Xie, P., Wang, Z., Ding, J., Wang, J., Wang, S., Wiesner, M.R., 2019. Comparative study on the pretreatment of algae-laden water by UV/persulfate, UV/chlorine, and UV/H<sub>2</sub>O<sub>2</sub>: variation of characteristics and alleviation of ultrafiltration membrane fouling. *Water Res.* 158, 213–226. <https://doi.org/10.1016/j.watres.2019.04.034>.
- Wang, H., Pampati, N., McCormick, W.M., Bhattacharyya, L., 2016. Protein nitrogen determination by kjeldahl digestion and ion chromatography. *J. Pharma. Sci.* 105, 1851–1857. <https://doi.org/10.1016/j.xphs.2016.03.039>.
- Wasyłka, J.P., 2018. A new tool for the evaluation of the analytical procedure: green Analytical Procedure Index. *Talanta* 181, 204–209. <https://doi.org/10.1016/j.talanta.2018.01.013>.
- Wheat Flour and Flour Testing Methods: A Guide to Understanding Wheat and Flour Quality WHEAT MARKETING CENTER, 2004. INC. Portland, Oregon USA.