Authentication of organically grown vegetables by the application of ambient mass spectrometry and inductively coupled plasma (ICP) mass spectrometry; The leek case study.

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Abstract

Health conscious and environmentally aware consumers are purchasing more organically produced foods. They prefer organic fruits and leafy vegetables as these are much less likely to have been exposed to contaminants such as pesticides.

The detection of fraudulent activity in this area is difficult to undertake, because many chemical plant protection treatments degrade very quickly or can be washed off to remove evidence of their existence.

It was found that when combining DART-MS with a compact, inexpensive and robust single quadrupole mass spectrometer, it was possible to differentiate organic from conventional

leeks with 93.8% to 100% accuracy. ICP-MS results showed similar performance, with an ability to differentiate conventional from organic leeks with 92.5% to 98.1% accuracy.

This study has paved the way for the certification of vegetables as being organically produced. The next step is to create data libraries to support the roll out of the methodologies described.

Keywords

DART, ICP-MS, elemental analysis, ambient mass spectrometry, food fraud, metabolomics

1. Introduction

The fresh fruit and vegetable sector features complex supply chains and a particularly short time window for detecting criminal or food safety issues. Vegetable shelf life can be a low number of days, so to minimise spoilage and to ensure a high quality product that is attractive to consumers, it needs to move rapidly from farm to consumer.

This requirement for speed means innovative solutions to accelerate distribution have been developed, and it is now normal to see products being packed and prepared for retail customers in the field (Minna, 2008). This approach does mean large quantities of fruit and vegetables never pass through markets and avoid distributors or resellers, thereby limiting potential points where testing could be undertaken in a central location, such as a warehouse (Minna, 2008).

The frequent absence of a convenient central location where more complex analytical techniques could have been housed not only makes portable techniques more desirable, but makes easier and less complex techniques especially desirable (Minna, 2008).

The retrospective analysis of fruit and vegetables in large food testing laboratories remains possible, but is increasingly undesirable as supply chains become more vulnerable to

fraudulent activity (S. M. van Ruth, Luning, Silvis, Yang, & Huisman, 2018). The demand for produce with the longest possible shelf life means items will often be packaged on farms and then combined with similar pre-packaged produce from neighbouring farms. This produce is then delivered directly to a retailer's distribution centre, avoiding markets and processing plants thereby allowing frauds perpetrated by farmers and distributors to more easily go undetected (Saskia M van Ruth & de Pagter-de Witte, 2020). The involvement of organised crime syndicates, who are very adept at counterfeiting documentation, can make deciphering the exact supply chain any given product follows even more complicated, particularly if mislabelling or substitution events occur (Moyer, DeVries, & Spink, 2017).

The types of testing which are required for organic fresh fruit and vegetables authenticity are those which are rapid, robust, low cost and thus enabling significant numbers of samples to be analysed over a short period of time.

The most widely used approach for many years has been to assess organic produce to determine whether it is free from chemical treatments (Pylypiw Jr, 1993). This approach is not fit for purpose, because combining the output from multiple farms means traces of chemical treatments can be transferred from a conventional product onto an organic product, whilst deliberate criminal activity may involve washing the produce. This is a particular area of concern with leafy vegetables which may already undergo washing to remove soil immediately post-harvest (Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015).

An alternative approach is to assess differences in the metabolomic and elemental profiles of the crop. The effect on the metabolomic and elemental profile of the product is long-lasting or permanent, and analysing the produce for these markers represents a potentially novel way of detecting fraudulent activity or confirming authenticity (Creydt & Fischer, 2020; Mihailova et al., 2021).

Leeks were chosen for this analysis as they are thought to be at a high risk of organic mislabelling fraud (Backer, Aertsens, Vergucht, & Steurbaut, 2009; Santana-Mayor, Socas-Rodríguez, Herrera-Herrera, & Rodríguez-Delgado, 2019).

Leeks also have desirable traits for this 'path finder' exercise, having a metabolomic profile which changes from the top leafs of the plant down to the root structure, accordingly, testing different parts of the leek, at least one part of the plant would show meaningful variation between organic and conventional production systems (Schmidt, Nyberg, & Staerk, 2014). It was similarly thought that the elemental composition of the leek would also vary from the leaf to the root, and again, at least one section would enable the distinction between organic and conventional production systems (Golubkina et al., 2020).

Two analytical mass spectrometry techniques were tested to assess whether they are capable, either independently of each other, or when taken together as complimentary techniques, of detecting fraudulent activity and confirming produce authenticity.

DART-MS, is one of the most rapid mass spectrometric measurement techniques currently available (Birse et al., 2020). Capable of testing individual samples in under one minute, this technique also offers the potential of portability and in-field testing when combined with a compact single-quadrupole mass spectrometer (Kenny & Whyatt, 2019). DART-MS can also be used with high-resolution platforms, making the technique especially flexible because the ion-source is available for almost all instrument manufacturer interfaces, and existing mass spectrometers can normally be adapted to operate as DART-MS systems (Cajka et al., 2013).

DART-MS was used for this study to build chemometric models based around the volatile molecular features that are distinct to organic and conventional products, enabling their differentiation.

ICP-MS is an elemental mass spectrometry technique, and like DART-MS, offers rapid sample testing capabilities (Bronzi et al., 2020). The technique, whilst typically confined to the

laboratory, can lead to alternative elemental analysis techniques being developed from the datasets generated with ICP-MS analysis, most often hand-held XRF (Worku et al., 2019).

ICP-MS for this project was used to develop chemometric models comparable in their nature with those developed from DART-MS data, to understand if the differentiation of organic from conventional leeks was achievable.

2. Materials and methods

2.1 Chemicals

Methanol (HPLC grade) was purchased from Honeywell Riedel-de Haen (Seelze, Germany), Nitric acid (67%-69%) was purchased from VWR (Lutterworth, UK), rhodium internal standard was purchased from Sigma-Aldrich (St Louis, MO, USA), multi-element certified reference materials (CLMS-2 and CLMS-4) were obtained from SPEX CertiPrep (Metuchen, NJ, USA) and instrumental tuning solution (5185-5959) was supplied by Agilent Technologies (Santa Clara, CA, USA). 18.2 MΩ de-ionised water was produced by a Millipore Integra 3 system (Merck-Millipore, Billerica, MA, USA).

2.2 Samples and sample preparation

Organically grown (n=40) and conventionally grown (n=40) whole leeks were sourced from trusted suppliers in Belgium. A further batch of organically grown (n=134) and conventionally grown (n=108) whole leeks were sourced from a mixture of the same and different trusted suppliers in Belgium. The leeks were maintained at a temperature of 4 °C from shortly after harvest to the point they were delivered to the laboratory. Three subsamples were taken from each leek; one from the top of the leaf, one from the centre of the stem and one from the point where the root attaches to the stem. The samples were then stored at -45 °C prior to further processing.

The frozen samples were homogenized using a compact kitchen food processor and stored in 50-mL centrifuge tubes (Sarstedt, Nümbrecht, Germany). The samples were then freeze-dried using a Lablyo freeze drier (Frozen in Time, York, United Kingdom) for two separate 24-hour periods. The dried material was then stored at -45 °C.

2.3 DART-MS analysis

A 10mg sample was weighed using a Discovery DV215CD balance (Ohaus Europe GmbH, Nanikon, Switzerland) into a 1.5 mL Eppendorf tube. 1 mL of methanol:water (1:1 v/v) was added to the tube and the sample vortexed briefly. The sample was then shaken using an Eppendorf Thermomixer C (Eppendorf, Hamburg, Germany) for 10 minutes at 2000rpm and 20 °C, before being centrifuged at 15,000 rpm for 10 minutes at 20 °C using a Rotina 380R benchtop centrifuge (Hettich, Tuttlingen, Germany).

A 10 µL aliquot of the supernatant was then spotted onto 12 position QuickStrips (IonSense, Saugus, MA, USA) and permitted to dry. Samples were then analysed using a Waters QDa Performance single-quadrupole mass spectrometer (Waters, Wilmslow, United Kingdom) onto which an IonSense DART SVP-201 ion source was mounted orthogonally by means of an IonSense Vapur interface.

The initial extraction was modified by reducing the solvent volume from 1 mL to 500 µL and then by trialling different solvents. Methanol, methanol:water (1:1 v:v and 9:1 v:v), ethyl acetate, methanol:ethyl acetate (1:1 v:v), acetonitrile (ACN), methyl tert-butyl ether (MTBE), 2-propanol (IPA) and methanol:IPA (1:1 v:v) were all trialled.

The extract spotted onto the QuickStrip cards was allowed to dry, subsequently, each card was passed through the plasma source orthogonally by means of a powered rail system operating at a speed of 2 mm/sec.

The Waters QDa Performance single-quadrupole mass spectrometer was configured to use a cone voltage of ±30 V with the source temperature set to 150 °C. Cone voltages between ±10 V and ±100 V were trialled and as expected, in-source fragmentation increased as the

cone voltage increased. The best compromise of reducing background interferences without excessive in-source fragmentation was ±30 V. The source temperature made only a small difference but increasing it from 120 °C to 150 °C reduced background interferences.

Samples were run in both positive and negative ionisation modes, with the positive ionisation mode providing spectra with more features.

Spectra were acquired in the mass range 100-1000 m/z, the scan rate was set to 0.5 seconds per scan, and the data was acquired in continuum mode for both positive and negative ionisation modes.

The DART SVP-201 source was set to use helium for the ionisation gas and nitrogen as the standby gas, the volume and pressure were set at the factory but are approximately 3.5 mL per minute at 8 bar. The temperature was set initially to 350 °C but during method development temperatures from 250 °C to 450 °C were trialled, and 350 °C was found to provide the best spectral features whilst minimising background interferences.

The grid voltage on the DART source was set to +350 V and -250 V and again this was found to provide the best compromise between spectral features and reducing background interferences.

Instrument control was undertaken using MassLynx 4.2 SCN 993 (Waters, Wilmslow, UK).

2.4 ICP-MS analysis

A 100 mg dry sub-sample was weighed using a Discovery DV215CD balance (Ohaus Europe GmbH, Nanikon, Switzerland) into metal free 50 mL centrifuge tubes (VWR, Lutterworth, UK). 2 mL each of 67-69% nitric acid and >30% hydrogen peroxide were added to the tubes, which were left in a fume hood overnight to facilitate sample digestion.

The sample was then microwave digested using a Mars 6 system (CEM, Matthews, NC, USA) with a 65 min digestion protocol. The samples were heated gradually to 95 °C over a 35 minute period, then held at 95 °C for a further 30 minutes. A rhodium internal standard was added

just prior ICP-MS analysis. The tubes were then made up to 30 g using Milli-Q deionised water for which a VWR SE622 balance (VWR, Leuven, Germany) was used.

Instrument calibration was achieved using a 9 point external calibration with blank offset for all elements. The calibration curve was prepared over the range 0.1, 1, 2.5, 5, 10, 20, 50 and 100 ng/mL, prepared from the SPEX Solution 2 and 4 certified multi-element standards. The calibrants in the range 10 ng/mL to 100 ng/mL were weighed out into metal free 50 mL centrifuge tubes on an Ohaus Discovery DV215CD balance, whilst calibrants in the range 0.1 ng/mL to 5 ng/mL were prepared by serial dilution of the 10 ng/mL standard. A calibration blank was also added to the worklist.

Control standards (10 ng/mL) were added to the start and end of each worklist, prepared from different batches of the SPEX calibration standards. All calibrants were prepared in 2% nitric acid (67-69%) in Milli-Q deionised water.

The samples were analysed using an Agilent 8900 (Model G3665A) triple-quadrupole ICP-MS (Agilent, Santa Clara, CA, USA). The ICP-MS x-Lens was configured for food applications and the plasma ignition was set for aqueous solutions. The instrument was configured in MS mode with the MS1 mass analyser disabled and helium was used as a collision gas to eliminate polyatomic interferences. Sample introduction was by a peristaltic pump connected to an Agilent MicroMist nebuliser and then to an Agilent SPS4 autosampler.

The Agilent 8900 ICP-MS-MS was configured in spectrum acquisition mode, with a point Q2 peak pattern of 3 replicates. Sweeps per replicate was set to 50.

Instrument control was undertaken using Agilent ICP-MS MassHunter 4.5 (G7201C, Version C.01.05) (Agilent, Santa Clara, CA, USA).

2.5 Data Processing

DART data was acquired using MassLynx 4.2 SCN993 and imported into Waters Abstract Model Builder (AMX) (0.9.2092.0) (Waters Research Centre, Budapest, Hungary) where the data was pre-processed using the standard MassLynx algorithms, background subtracted and the total ion current (TIC) was normalised. AMX generated a matrix of normalised TIC values, and no TIC threshold was used for this. Mass binning was undertaken using AMX and a bin size of 1Da was chosen, resulting in 900 mass bins. The resulting matrix file was exported as a CSV format file. This process was repeated for all three sections of the leeks in positive ionisation mode, and then repeated again fully for all three sections of the leek in negative ionisation mode, generating a total of six models.

ICP-MS data was acquired using Agilent ICP-MS MassHunter 4.5 and the data was then processed using Agilent's Online ICP-QQQ software to generate a matrix of elemental concentrations in parts per billion (ppb). Background subtraction was performed using blank samples as a reference. The matrix file was then exported in CSV format, and Log10 transformed for all values, before silicon and sulphur were removed as background subtraction since these elements were not sufficiently reliable, and the dataset was then subjected to statistical analysis.

The resulting matrix files from both DART and ICP-MS were imported into SIMCA 17.0 (Sartorius Stedim Biotech, Umea, Sweden). The DART data was mean-centred and grouped into four classes per model, either organic or conventional leaf/stem/root, blank and background classes. The data was UV scaled.

The Log10-transformed ICP-MS data was grouped into eight classes, three classes relating to organic leaf, stem and root, a further three for conventional leaf, stem and root, with a QC class and a calibration standard class. The data was UV scaled as with DART.

The workflow for both DART and ICP-MS was then substantially identical; initially Principle Component Analysis (PCA) was performed on the datasets. PCA is an unsupervised

modelling technique which can help to ascertain if there are any inherent trends within the dataset without trying to find trends related to specific classes.

Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) supervised models were generated, OPLS-DA models will find differences between specific classes, making them useful as a way to find greater differences between classes than may be immediately obvious from PCA modelling.

The model validity was determined by recording the R^2, Q^2 and Root Mean Squared Error of Cross Validation (RMSECV) values. R^2 is used to describe variation across the model, as described by the number of components within the model, Q^2 is used to describe the overall predictive capability of the model, without relying on the data used to train the model. Permutation testing was performed to assess whether the OPLS-DA models were over-fitted for the training dataset, 500 permutations were performed for this purpose.

3. Results

3.1 DART-MS results

The PCA models showed clear evidence of separation between the organic and conventional leeks, this was seen (Fig. S1a-l) in all subsamples taken from each plant, and in both positive and negative ionisation modes. The separation appears to be relatively consistent throughout the leek, with similar separation evidenced in the leaf, stem and root PCA plots, and in both the first batch (Fig. S1a-f) and second batch (Fig. S1g-l). It was also observable when combining the two datasets that there was some level of temporal shift between batches (Fig. S2a-f).

The first OPLS-DA models to be built for batch 1 were the leaf models, using positive and negative ionisation mode datasets. The cross classification rates for the batch 1 models ranged from 95.0% to 100% (Table 1) with the leaf models showing the greatest correct

classification performance. The R^2 and Q^2 values (Table S4a) for these models was consistent with their correct classification rate. The model R^2 values, which assess the data fit within the model varied from 0.762 for the stem negative model to 0.954 for the leaf negative model, the Q^2 values, which assess the predictive ability to the model, varied from 0.532, also for the stem negative model to 0.814 for the root positive model.

The second batch of samples were analysed in the same way, and for their corresponding models, the correct classification rate ranged from 93.8% to 100% (Table 2) with the leaf models again showing the greatest correct classification performance. The model R^2 and Q^2 values (Table S4b) for these models was again consistent with their correct classification rate. The R^2 values varied from 0.698 for the stem negative model to 0.889 for the root positive model, the Q^2 values varied from 0.350, also for the stem negative model to 0.870, again for the root positive model.

Variable Importance for the Projection (VIP) data from two-class OPLS-DA models were generated and those mass bins which were most responsible for the separation between the two classes were listed in four tables (Tables S1a-d) with a positive and negative ion table for each batch. A cut-off value of two was applied to view the most significant mass bins.

The limitations of the instrument in relation to resolution, scan speed, longer-term stability and the single-quadrupole architecture in general, combined with the variety of different potential adducts formed by the DART ionisation source made tentative identification of the compounds within each of these mass bins impossible. The VIP data remains of interest as it shows some of the compounds most responsible for separation are found solely in one part of the leek, other compounds are found in one part and the adjacent section, whilst others can be found throughout the leek.

Tentative analysis suggests these compounds are plant sterols, but no formal identification was attempted in light of the limitations of the single-quadrupole instrument configuration and inability to accurately assess ionisation mechanism and adduct formation within the source.

3.2 ICP-MS Results

PCA models for the leaf, stem and root were prepared as previously described using SIMCA 17.0. The PCA models (Fig. S3a-c) show very little separation for the stem, but better separation for the leaf and root models.

The OPLS-DA models showed a correct classification rate of 98.1% for the leaf, 92.5% for the stem and 96.5% for the root (Table 3).

The R^2 values for these models were 0.815, 0.674 and 0.745 respectively, whilst the Q2 values were 0.717, 0.614 and 0.674, showing the model built around the leaf data to be both the model with the highest level of fit and the greatest predictive ability.

Technical issues with the analysis resulted in no data being returned for 9 samples.

Variable Importance for the Projection (VIP) data from OPLS-DA models were generated and the elements ranked from highest to lowest in relation to their impact on separation (Tables S2a-c) for the leaf, stem and root.

Statistical analysis of the dataset was also performed, reviewing the minimum, maximum and median concentrations, along with the standard deviation for each element, and comparing the different levels present in the leaf, stem and root for conventional and organic classes (Table S3).

4. Discussion

The DART-MS and ICP-MS techniques have both been shown to deliver precise and accurate identification of the production systems used in the production of leeks. This is most likely attributable to discreet differences between the metabolomic pathways of those plants which are given artificial prophylactic plant protection treatments, and those which were not. It is likely that the organic plants are to be under more predation stress, a result of having limited and less effective artificial plant protection treatments available (Brandt & Mølgaard, 2001).

The outcome of this study is particularly relevant given that the conventional and organic leeks involved were representative retail samples taken from an existing supermarket supply chain, rather than relying on a study which made use of specially cultivated leeks for the express purpose of attempting to differentiate between organically grown and conventionally grown leeks.

Initial sample preparation was consistent with that required for liquid or gas chromatography and mass spectrometry techniques, such as ultra-performance liquid-chromatography high-resolution mass spectrometry (UPLC-HRMS) or gas chromatography-mass spectrometry (GC-MS). The typical steps of freeze-drying, homogenisation, simple liquid-liquid extractions and dilutions were still undertaken prior to DART and ICP analysis, however other steps, such as sample clean-up, solid-phase extraction (SPE), sample concentration and reconstitution were avoided, potentially saving a significant amount of time and cost through the removal of SPE cartridges, filters and additional solvents. The removal of any chromatography also saved both time and reduced costs in comparison to LC or GC based approaches.

Freeze-dryer capacity was found to be a limitation, but with a total of 110mg of material being needed between the two separate analysis, vastly reduced sample sizes could be used, allowing many hundreds of leek samples to be freeze-dried per 48-hour cycle, and if DART-MS was to be used alone, requiring just 10mg of dried material, many thousands of samples could be dried each cycle.

The extraction for DART-MS took a few seconds per sample, significantly faster than any comparable sample preparation for LC-MS, which would typically require filtration and transfer into either a 96-well plate or appropriate LC vials, a process adding both cost and time. The QuickStrip used in the DART-MS is comparable in cost to glass vials used in existing analytical techniques.

The DART-MS sample analysis time was 30 seconds per sample, with each section of leek being analysed in duplicate, and in both polarities, giving a gross analysis times of 2 minutes for each section of leek, and of 6 minutes per leek in total. This is extremely rapid when compared with traditional UPLC-HRMS; previously described work on leeks and related species have used liquid chromatography runs lasting almost 30 minutes as an example (Creydt, Arndt, Hudzik, & Fischer, 2018). There are further performance gains to be had when comparing DART with gas chromatography, where 1-hour analyses are not uncommon.

The complex ionisation mechanisms, in particular the secondary ionisation mechanisms found within the DART-MS ion source, also support the suggestion that small atmospheric changes can substantially impact ionisation performance, and ultimately, instrument sensitivity and stability (Guo et al., 2017). This was found to be the case with this study, however work has been undertaken in recent years to improve the behaviour of the DART ion source, and recent publications have suggested almost all of the speed and ease of use benefits of the source can be maintained whilst the source is closed off in some way to protect it from significant changes in atmospheric conditions, resulting in dramatic improvements in instrument sensitivity and stability (Kloth, Khanipour, Mayrhofer, & Katsounaros, 2021).

Methanol:water (1:1 v:v) gave the best compromise of background noise and spectral features, further optimisation and different solvent combinations may also aid in the reproducibility and reduction of background noise, similarly the use of dopants such as ammonia is recognised as enhancing the DART ionisation process and may warrant further investigation in any future work.

The performance of the DART-QDa platform also confirms that it is possible to achieve robust, reliable and relatively reproducible differentiation between organic and conventional leeks using a compact, low-resolution instrument. The Waters DART-QDa package, which makes use of the same QDa Performance mass spectrometer and IonSense SVP source, is of particular interest here. The package includes a compact and relatively inexpensive mass spectrometry based platform, and an integrated chemometric software package, LiveID, which enables end-users to easily and quickly generate a spectral library against which leeks of unknown origin could be analysed.

The system is well suited to the smaller routine testing laboratories found in the agri-food and environmental analysis sectors, whilst the software applications included are tailored for use by the less experienced user, making it extremely well suited to routine analysis work in areas where high throughput, low profit margin products are routinely tested.

ICP-MS was the other technique used to analyse leeks, exploiting the freeze-dried samples that were prepared for DART-MS analysis. This technique can make use of more simple and rapid sample preparation techniques; vegetable sections can be digested directly without the need for freeze-drying and homogenisation, which would reduce analysis time by many days for routine analysis and many weeks for large studies.

The use of ICP-MS for elemental fingerprinting is a more recent application of the technology, and is an excellent application for lower cost single-quadrupole instruments, instead of the triple-quadrupole instrument used in this study; to emulate the performance of a single-quadrupole instrument, the Agilent 8900 used for this study was configured into MS mode and operated with the Octapole Reaction System (ORS) in collision cell using default collision gas, helium, which was used solely to eliminate any polyatomic interferences. The use of the ORS in reaction mode, which can be used with a number of different gases to generate specific

polyatomic species, was not considered appropriate for this study as it would have interfered with the elemental fingerprint generated, accordingly the ORS was otherwise left disabled.

The sample analysis time for ICP-MS is also highly attractive and combined with large auto-samplers, such as the Agilent SPS4 system fitted to the Agilent 8900 ICP-MS-MS used for this study, it is possible to rapidly analyse hundreds of samples in a relative short period of time, thereby enabling the creation of large and complex chemometric models, which can hopefully differentiate between different production systems.

This technique demonstrated several key advantages over DART-MS for the analysis of leeks. First, ICP-MS showed no signs of a temporal shift, especially when comparing the reference and QC materials in a PCA plot, because they show no signs of separating into the two batches (Fig. S4) which would make the long term analysis considerably more robust and less complex to manage. ICP-MS also benefits from the ability to run both internal and external standards together with the availability of a wide range of matrix-matched reference materials, the management of any temporal shift which may emerge over longer periods should be considerably easier to manage with ICP, and whilst work is being undertaken to use internal standards with DART-MS, this frequently relies on more expensive labelled standards (Nei, Nakamura, Ishihara, Kimura, & Satomi, 2017).

The results show that modelling behaviour is dependent on small differences across all of the elements being analysed, rather than any one element in particular. This behaviour would appear to confirm that separation is not directly related to crop protection products employed. Copper, which is widely used as a fungicide in organic produce, was only the sixth most important element for separation in the leaf model.

There were minor difficulties encountered with the analysis of the leeks using ICP-MS. The leeks that were supplied by a large supermarket chain in Belgium, were all washed and in some instances, were also pre-packaged, despite this, there was still considerable soil and other organic detritus on the leeks. The root structure had the largest concentrations of soil, but it was present across most of the leeks, accordingly care was taken to remove as much of this as possible prior to sectioning and freeze-drying, which was time consuming and added to the demands on sample preparation for analysis. Rinsing the leeks in deionised water was considered but to retain any trace evidence of crop protection products, was discounted.. The presence of soil is thought to have resulted in a small number of samples encountering technical difficulties during analysis, with the peristaltic pump tubing becoming blocked during analysis.

The modelling performance for both DART-MS and ICP-MS was broadly comparable and interestingly, the sections of the leek where DART-MS performed best corresponded closely to the sections of the leek where ICP-MS also performed most strongly i.e. the leaf. The stem was the poorest performing section for both techniques, and the modelling performance for the root was typically second to the leaf for both DART-MS and ICP-MS analysis.

The use of the leaf rather than the stem or root could make routine analysis using either of these two techniques considerably easier for producers; whilst analysing the root or stem would require all of the leek to be sacrificed for analysis, leeks routinely have their leaves trimmed to make them a consistent length and height for packaging and display purposes. It would be possible to cut a small section of leaf from each leek, or use the off-cuts produced, this would reduce the overall cost of this testing and reduce food wastage.

ICP-MS may not be amenable to a portable on-site testing configuration, but it is likely that it will be possible to make use of the ICP-MS in a laboratory setting to help with the development of handheld elemental analysis technologies, such as XRF, by using the technique to build, calibrate and assist in the validation of chemometric models for XRF analysis (Syta, Kępa, Mistewicz, Wesołowska, & Wagner, 2018).

Finally, for both techniques, additional applications need to be considered – few if any routine testing laboratories would be happy with an instrument which can only conduct one type of analysis for one vegetable. DART-MS has a lengthy history of being used to test for contaminants, particularly volatile contaminants such as pesticides, and whilst this approach wasn't leveraged for this study, the analysis of organic produce for the presence of illicit pesticides or other crop protection products is routinely undertaken with DART-MS (Ellis et al., 2015).

ICP-MS is similarly wide-reaching in its potential applications – extensively used to analyse soil, water and other environmental samples, it has great potential in testing for the use of unauthorised fertilisers and soil condition modifiers. It can also test for elements which may be hazardous to health in higher concentrations, as is the case with arsenic in rice (Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002). ICP-MS is also additionally used in more recent work to develop geographic models which can indicate the area in which a crop is grown. This, whilst not directly relevant to differentiating between conventional and organic crops, can nevertheless be a highly effective way of detecting mislabelling frauds and tracking the origins of such produce (Worku et al., 2019).

The use of DART-MS and ICP-MS to confirm the authenticity of leeks demonstrated the potential to use a choice of mass spectrometry platforms, however for those products which display less clearly demonstrable separation, the combination of full or partial datasets from the two techniques may provide an additional opportunity to conclusively prove authenticity, albeit under penalty of additional cost in provisioning two instruments and additional time in running two very different analytical methods.

5. Conclusion

DART-MS and ICP-MS are two very different mass spectrometry platforms, but both were able to differentiate between conventionally and organically grown leeks. The two platforms have broadly similar chemometric modelling performance and little to separate them in terms of sample preparation time and possible instrument throughput.

The two techniques are capable of high throughput and demonstrate considerable potential for the routine confirmation of production systems used in vegetable production.

The choice of which technique to implement is likely to depend on factors external to the instrumentation discussed here; existing instrumentation, operator experience, supplier preference, laboratory capabilities, additional analysis work to be undertaken and ultimately, cost.

ICP-MS arguably provides the higher performance system for analysis, with relatively simple sample preparation, an auto-sampler which enables high sample throughput for routine analysis, and the ability to use calibration standards, certified reference materials and matrix blanks to allow the performance of the ICP system to be monitored and adjusted in a way to make measurements extremely repeatable and consistent over many months or years, in a way that it is not possible to undertake with DART-MS.

ICP-MS would fit well with testing laboratories that also undertake elemental analysis of materials, such as soil and water samples, trace element analysis and other similar work, complimenting many of the typical types of analysis work undertaken by routine agri-food and environmental testing laboratories.

DART-MS as a general technique, would be a good fit for laboratories undertaking molecular analysis, particularly in the area of food safety and fraud detection, such as residue and contamination analysis, mycotoxin screening or fraud analysis, and can avoid the need for an entirely new mass spectrometer, being compatible with instruments from multiple vendors including Agilent, SCIEX and Thermo, as well as Waters.

DART-MS also provides one of the fastest analysis options available, with a capability to screen many thousands of samples per day using cost-effective consumables in a process which can be dramatically simplified for non-expert mass spectrometry users.

This study has therefore shown that it will be possible, going forward, to verify leafy green vegetables as being organically produced, to do this, large libraries of DART and ICP data will need to be produced and processed, with the possibility of fusing those libraries from different instruments together as a way of generating additionally robust and reliable models.

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