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1 Authentication of organically grown vegetables by the application of ambient mass spectrometry
2 and inductively coupled plasma (ICP) mass spectrometry; The leek case study.
3

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16

17 **Abstract**

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

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

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

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

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

30

31 **Keywords**

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

33

34 **1. Introduction**

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

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

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

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

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

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

62

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

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

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

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

85

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

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

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

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

101 laboratory, can lead to alternative elemental analysis techniques being developed from the
102 datasets generated with ICP-MS analysis, most often hand-held XRF (Worku et al., 2019).
103 ICP-MS for this project was used to develop chemometric models comparable in their nature
104 with those developed from DART-MS data, to understand if the differentiation of organic from
105 conventional leeks was achievable.

106

107 **2. Materials and methods**

108 **2.1 Chemicals**

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

116 **2.2 Samples and sample preparation**

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

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

129 **2.3 DART-MS analysis**

130 A 10mg sample was weighed using a Discovery DV215CD balance (Ohaus Europe GmbH,
131 Nanikon, Switzerland) into a 1.5 mL Eppendorf tube. 1 mL of methanol:water (1:1 v/v) was
132 added to the tube and the sample vortexed briefly. The sample was then shaken using an
133 Eppendorf Thermomixer C (Eppendorf, Hamburg, Germany) for 10 minutes at 2000rpm and
134 20 °C, before being centrifuged at 15,000 rpm for 10 minutes at 20 °C using a Rotina 380R
135 benchtop centrifuge (Hettich, Tuttlingen, Germany).

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

141 The initial extraction was modified by reducing the solvent volume from 1 mL to 500 µL and
142 then by trialling different solvents. Methanol, methanol:water (1:1 v:v and 9:1 v:v), ethyl
143 acetate, methanol:ethyl acetate (1:1 v:v), acetonitrile (ACN), methyl tert-butyl ether (MTBE),
144 2-propanol (IPA) and methanol:IPA (1:1 v:v) were all trialled.

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

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

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

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

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

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

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

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

168 **2.4 ICP-MS analysis**

169 A 100 mg dry sub-sample was weighed using a Discovery DV215CD balance (Ohaus Europe
170 GmbH, Nanikon, Switzerland) into metal free 50 mL centrifuge tubes (VWR, Lutterworth, UK).
171 2 mL each of 67-69% nitric acid and >30% hydrogen peroxide were added to the tubes, which
172 were left in a fume hood overnight to facilitate sample digestion.

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

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

178 Instrument calibration was achieved using a 9 point external calibration with blank offset for
179 all elements. The calibration curve was prepared over the range 0.1, 1, 2.5, 5, 10, 20, 50 and
180 100 ng/mL, prepared from the SPEX Solution 2 and 4 certified multi-element standards. The
181 calibrants in the range 10 ng/mL to 100 ng/mL were weighed out into metal free 50 mL
182 centrifuge tubes on an Ohaus Discovery DV215CD balance, whilst calibrants in the range 0.1
183 ng/mL to 5 ng/mL were prepared by serial dilution of the 10 ng/mL standard. A calibration
184 blank was also added to the worklist.

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

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

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

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

198

199 **2.5 Data Processing**

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

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

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

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

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

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

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

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

237

238 **3. Results**

239 **3.1 DART-MS results**

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

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

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

255

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

263

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

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

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

278

279 **3.2 ICP-MS Results**

280 PCA models for the leaf, stem and root were prepared as previously described using SIMCA
281 17.0. The PCA models (Fig. S3a-c) show very little separation for the stem, but better
282 separation for the leaf and root models.

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

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

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

289

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

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

297

298 **4. Discussion**

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

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

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

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

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

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

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

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

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

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

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

367

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

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

377

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

383

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

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

399

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

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

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

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

427 Finally, for both techniques, additional applications need to be considered – few if any routine
428 testing laboratories would be happy with an instrument which can only conduct one type of
429 analysis for one vegetable. DART-MS has a lengthy history of being used to test for
430 contaminants, particularly volatile contaminants such as pesticides, and whilst this approach
431 wasn't leveraged for this study, the analysis of organic produce for the presence of illicit
432 pesticides or other crop protection products is routinely undertaken with DART-MS (Ellis et al.,
433 2015).

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

443

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

450

451 **5. Conclusion**

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

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

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

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

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

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

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

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

484

485

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