Provenance confirmation of farmhouse cheeses produced on the island of Ireland

A Technology Viability Study

be safe be healthy be well

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Glossary of terms and abbreviations

IoI	Island of Ireland
RoI	Republic of Ireland
NI	Northern Ireland
GB	Great Britain
ICP-MS	Inductively coupled plasma mass spectrometry
IRMS	Isotope-ratio mass spectrometry
LOD	Limit of detection
ANOVA	Analysis of variance
PC	Principal component (score)
RSD	Relative standard deviation
IHS	In-house standard
QC	Quality control (sample)
PLS	Partial least squares regression
SIMCA	Soft independent modelling of class analogy
IAEA	International Atomic Energy Agency
USGS	United States Geological Survey
Ca	Calcium
Na	Sodium
Mg	Magnesium
P	Phosphorus
K	Potassium
Mn	Manganese
Cu	Copper
Se	Selenium
Fe	Iron
Mo	Molybdenum
Zn	Zinc
Bi	Bismuth
Ge	Germanium
Ln	Lanthanum
Sc	Scandium
Tb	Terbium
Y	Yttrium
Re	Rhenium
Ga	Gallium
Li	lithium
Ni	Nickel
U	Uranium
Sr	Strontium

Acknowledgements

This is a technical report of a research project funded by ***safe*food** to investigate the feasibility of applying specific analytical methods to the development of elemental and isotopic fingerprints for artisan cheeses produced on the island of Ireland. The goal of the research was to scope out the potential for this analytical approach to lead to the development of a form of provenance assurance that would provide a guarantee of quality thereby enhancing consumer confidence and strengthening the industry. The research was undertaken by Prof Gerard Downey, Principal Research Officer at the Teagasc Food Chemistry and Technology Department at Ashtown Food Research Centre, Dublin in association with Prof Chris Elliott, Director of the Institute for Global Food Security at Queen's University Belfast. Thanks are due to their respective research teams and also to the Project Management Board (see Appendix 1) for their input at the outset of the project. These include representatives of the Food Safety Authority of Ireland and Food Standards Agency Northern Ireland, the Consumers Association of Ireland, the Department of Agriculture, Food and the Marine, Cáis – the Association of Irish Farmhouse Cheesemakers, and the Istituto Agrario di San Michele all'Adige (IASMA). Thanks also to Sheridan's Cheesemongers for sourcing and supplying the majority of the island of Ireland artisan cheeses used in the project.

Executive Summary

This project was a technology viability study that set out to investigate the feasibility of applying specific analytical methodologies for isotopic and elemental analysis for identifying the provenance of artisan foodstuffs. Specifically, the research investigated if artisan cheeses labelled as produced on the island of Ireland (IoI) could be described as such. The strategy pursued generated a considerable quantity of baseline analytical data on the elemental and isotopic composition of IoI artisanal cheese as well as a selection of artisanal cheeses from Great Britain (GB) and mainland Europe. While it was not possible to confirm the geographic provenance of IoI artisanal cheeses with 100% accuracy, nonetheless trends in some of the data, especially the isotope data, suggest the possibility of effective segregation of IoI from mainland European, if not GB, cheeses. Therefore the analytical methodologies investigated have been scoped out for this purpose and can now be taken forward and applied in more focussed investigations involving artisan cheeses and other foods produced on the IoI.

Table of contents

Introduction and background

Provenance of processed foods is a significant quality attribute for many consumers and one for which they are willing to pay a price premium. As a consequence, the fraudulent mislabelling or adulteration of high-value foods now occurs on a global scale. Regulatory authorities and food businesses are focussing greater efforts in combatting food fraud which can have serious ramifications for both revenue and reputation. A number of provenance verification schemes have been established in other countries with the express purpose of protecting the denomination of quality associated with particular food products. This includes the Grana Padano or Parmigiano-Reggiano protected designation of origin status for artisan cheeses in Italy. There is currently no such scheme for artisan or 'farmhouse' cheeses produced on the IoI and yet it is desirable to facilitate a system of provenance confirmation which can provide confidence to consumers in the true geographical origin of artisan cheeses branded as produced on the IoI. It is therefore prudent at this point in time to investigate analytical methods that could be applied to provide consumers with the necessary assurance of the claimed IoI origin of such products.

The concentration and relative ratios of key analytes in a food products such as cheese are mainly influenced by animal diet and geographic location. Several reports from other countries or regions have shown that the use of multivariate analysis of analytical data comprising elemental and isotopic ratio values can provide confirmation of claimed geographic provenance. Given that food animals on the IoI are largely fed a grass-based diet and reside within a discrete insular geographical area, there is potential for developing robust fingerprint models that can characterise indigenous farmhouse cheeses.

Ultimately, the development of robust models will require the demonstration of two properties: (a) models should correctly classify the provenance of all IoI-produced artisan cheeses as originating on the IoI, and (b) models should correctly identify that farmhouse cheeses produced outside the IoI are not of IoI provenance. These two objectives are inseparable in the context of the provenance testing desired and must be demonstrated before any such model can be confidently used in practice. However, before this juncture is reached the application of analytical methodologies for the purposes of robust fingerprinting must be investigated.

Project aims and objectives

Objective 1

A database of farmhouse cheeses to be sampled was established. This took into account variables such as milk source, pasteurised vs. raw, hard vs. soft, cultured or not, etc. The list was compiled on the basis of discussions with members of the Project Advisory Group (Appendix 1) and other relevant bodies. Samples of each cheese type were taken at two time points during the project lifetime to include any possible seasonality effects on cheese composition.

Objective 2

Analytical procedures for both the inductively coupled plasma mass spectrometry (ICP-MS) and isotope-ratio mass spectrometry (IRMS) techniques were developed and validated.

Objective 3

Analytical data was collected on all samples. The data was subjected to univariate statistical analysis and a dedicated chemometric software package ('Unscrambler') was utilised for multivariate model development. Both discriminant and class modelling approaches were investigated. A databank of these data has now been created and stored at the Teagasc-Ashtown Food Research Centre.

Objective 4

Dissemination of the outcomes of the research to cheesemakers and other industry groups. This happened at a ***safe*food** Knowledge Network event on food safety in Cork in April 2015.

Key Project Recommendations

1. The analytical approach enabled the development of indicative models for confirmation of geographic provenance in artisan cheeses produced on the IoI. This will provide the basis for a more extensive study of the application of isotopic and elemental fingerprinting for this purpose.

2. Future investigations should incorporate a more extensive list of elements including transit5ion elements and δ 34S isotope measurements.

3. The development of robust identification models for IoI artisan cheeses will necessitate, in addition to the analysis of a greater number of elements, sampling over a greater geographic region and time span, as well as the application of alternative multivariate data analysis procedures.

4. A comprehensive data bank of IoI, GB and European artisan cheeses has been generated which could provide the raw material for further investigations to augment existing data.

Cheeses analysed in this study

Cheese samples

A representative range of IoI artisan cheeses was identified using published information on cheese producers and verbal contacts with a major cheesemonger (Sheridan's). In order to maximise the variance and capture any seasonality effects in this sample set, cheeses were obtained on two separate occasions during the project i.e., November 2013 and April 2015. Non-IoI cheeses were purchased in retail outlets in NI on several occasions during the project. The cheeses sampled included hard and soft, fermented and non-fermented, bovine, caprine and ovine; some also contained added non-traditional ingredients such as herbs, alcohol, etc.

In total, 131 samples of cheese were collected.

	Cow	**Sheep**	**Goat**	**Buffalo**
IoI	34R 40P 1U	7P	2R 9P 5U	
Great Britain	6P 14U	1U	1P 1U	
France	2R			
Germany	1P			
Greece		1U		
Italy	1R 1U			1U
Norway	1R			
Switzerland	2R			

R: raw; P: pasteurised; U: unknown

Care was taken to ensure that a wide geographical range of locations on the IoI was factored into the sampling plan, including proximity to the coast. IoI cheese samples were collected in counties Clare (5), Cork (31), Derry (1), Down (4), Galway (6), Kerry (2), Kilkenny (6), Limerick (7), Louth (4), Tipperary (10), Tyrone (5), Waterford (13) and Wicklow (4). The density of artisan cheese producers on the IoI increases significantly from North to South.

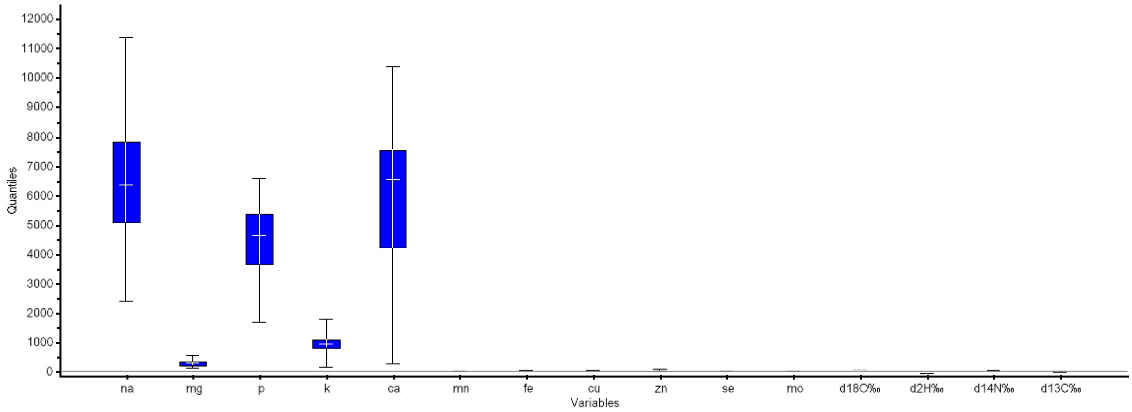
A full description of the analytical process is available in Appendix 2.

Research findings

The following parameters may give some preliminary indication of the potential for the analytes to be used in provenance determination.

Univariate analysis of mean results

Each of the mean results was examined to calculate overall mean, dispersion and normality (see Appendix 3). Gaps in the analytical dataset were encountered for certain elements i.e. Fe (5), Cu (25), Se (12) and Mo (5) (Appendix 4). This was due to technical difficulties encountered or the fact that the results obtained were at or below the limit of detection. Also, considerable differences were recorded for (a) elements: lowest (Mo; mean of 0.10 ppm) to highest (Na; mean of 6417 ppm), and (b) isotopes lowest (δ 14N; mean 6.5 ‰) to highest (δ 2H; mean 102.4‰).



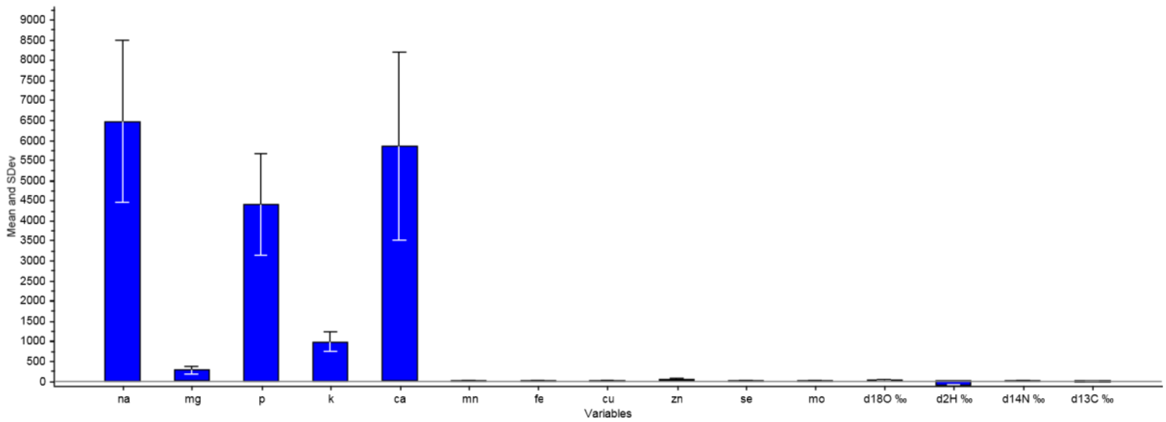


Figure 1. Graphical illustration of mean, maximum, minimum, range and standard deviation values for all analytes and all samples.

Given that the purpose of these analyses was to investigate whether any or all of the results could discriminate between cheese produced in or outside the IoI, it can be instructive to compare the indicators shown in Figure 1 for each analyte on the basis of samples grouped according to their provenance on (1) the IoI, (2) GB, and (3) mainland Europe. Figure 1 is a graphical display of the mean, maximum, minimum, range and standard deviation values listed in Appendix 4. The variation in magnitude of the results across the range of analytes is very clear. Because inter-correlation between any of the variables can pose problems with their use in certain data analyses, an examination of this inter-correlation was carried out (Appendix 5). It is clear that five elements – Mg, P, Ca, Zn and Mo – were inter-correlated, as were δ 18O and δ 2H.

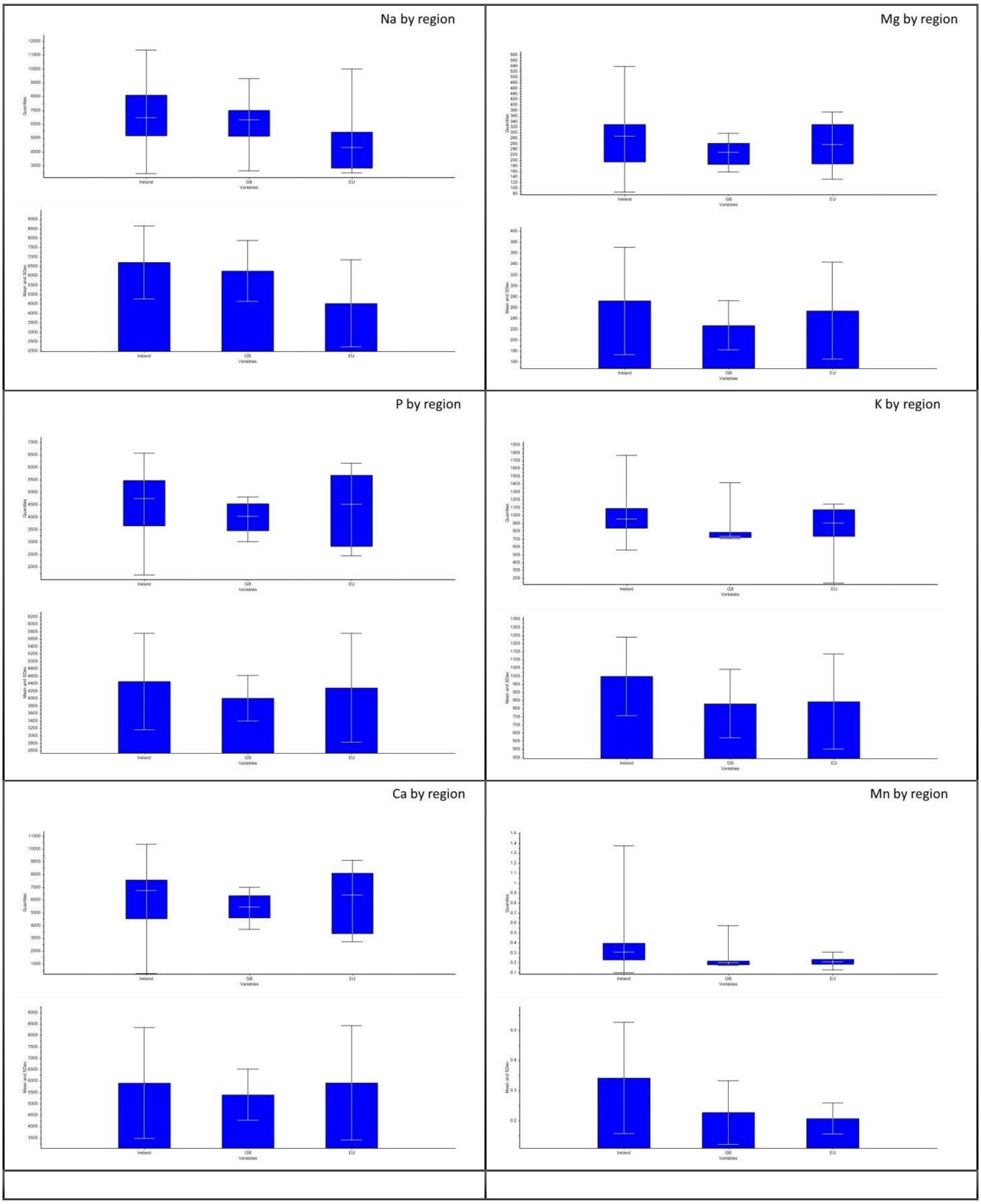


Figure 2. Summary comparison of elemental statistical descriptors on the basis of geographic region (IoI, GB and mainland Europe).

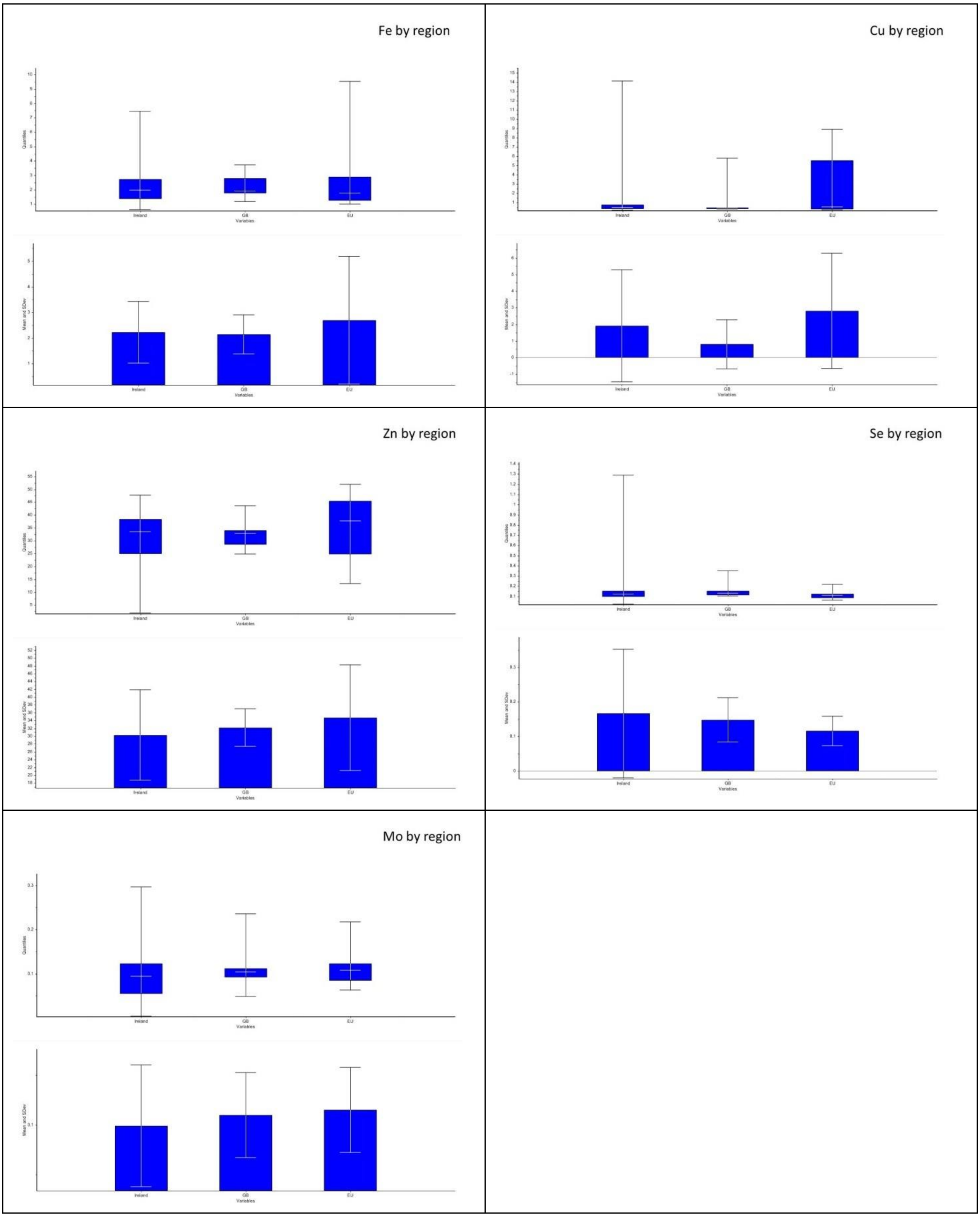


Figure 2 continued

Figures 2 and 3 also illustrate the comparisons between IoI, GB and mainland Europe. On the basis of the overlapping nature of all of the individual analytes, it may be concluded that no single element or isotope ratio is able to achieve the desired discrimination. Only in the case of δ 18O is there the possibility of some degree of separation between cheeses produced on mainland Europe versus those with an IoI or GB geographical provenance.

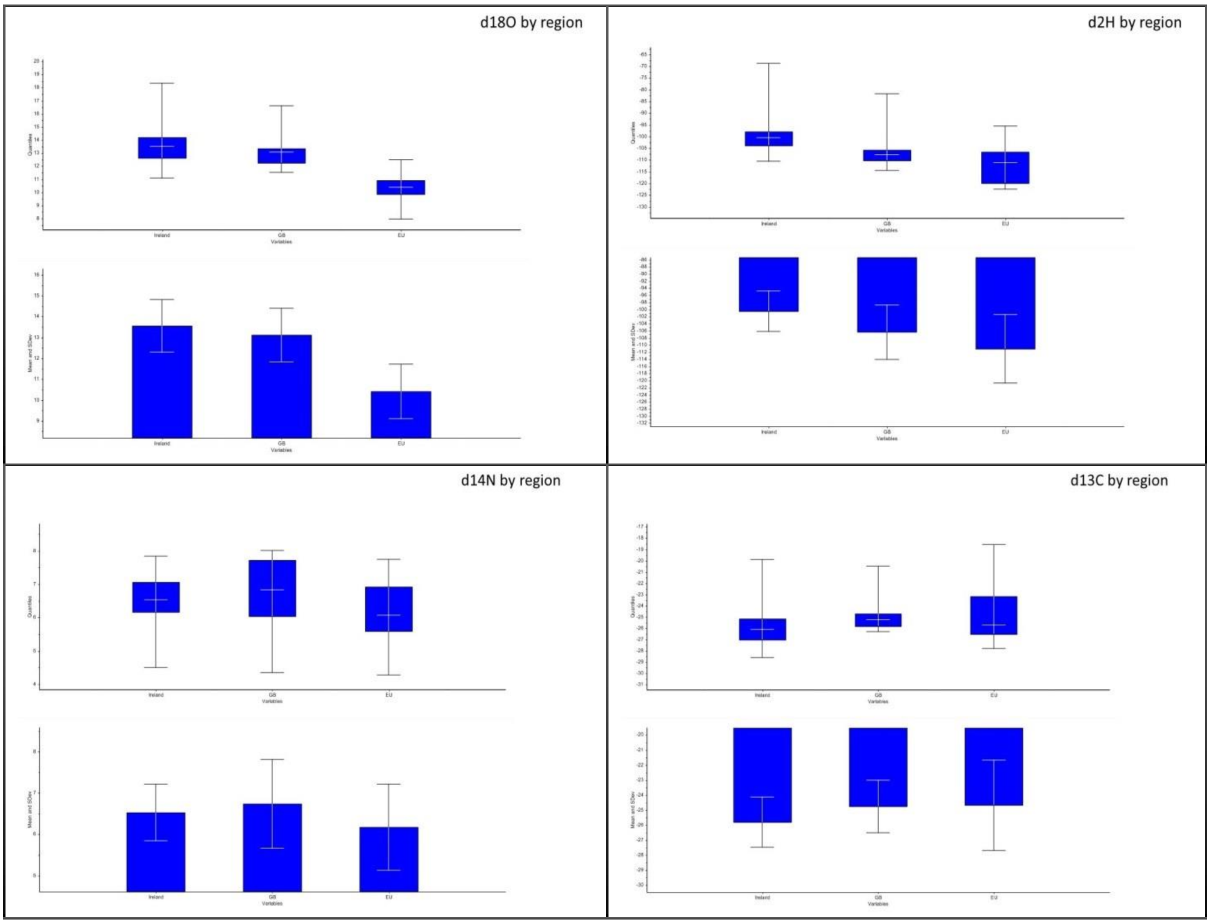
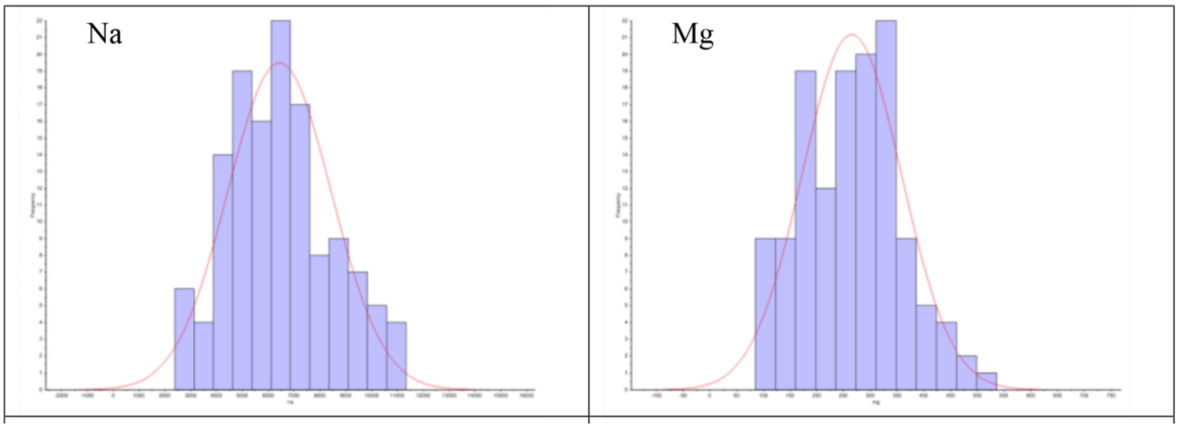


Figure 3. Summary comparison of isotopic statistical descriptors on the basis of geographic region (IoI, GB and mainland Europe).

Information on the dispersion or spread of individual analytes is also valuable for detecting any unusual results or distributions. Non-normal distributions can impact on many multivariate analytical procedures and may require data transformations to alter such distributions before further analysis. Histograms for each elemental analyte are shown in Figure 4.



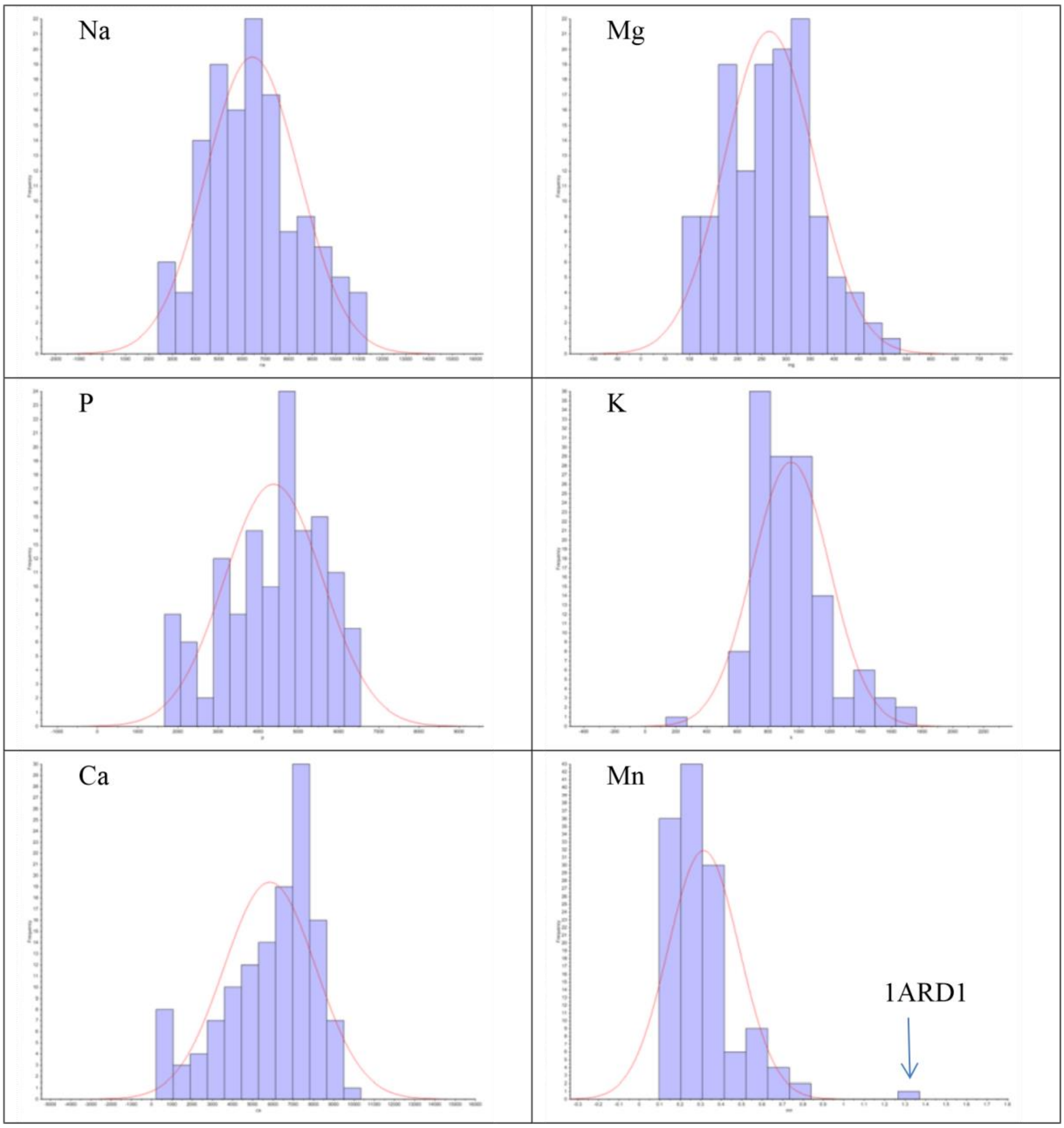
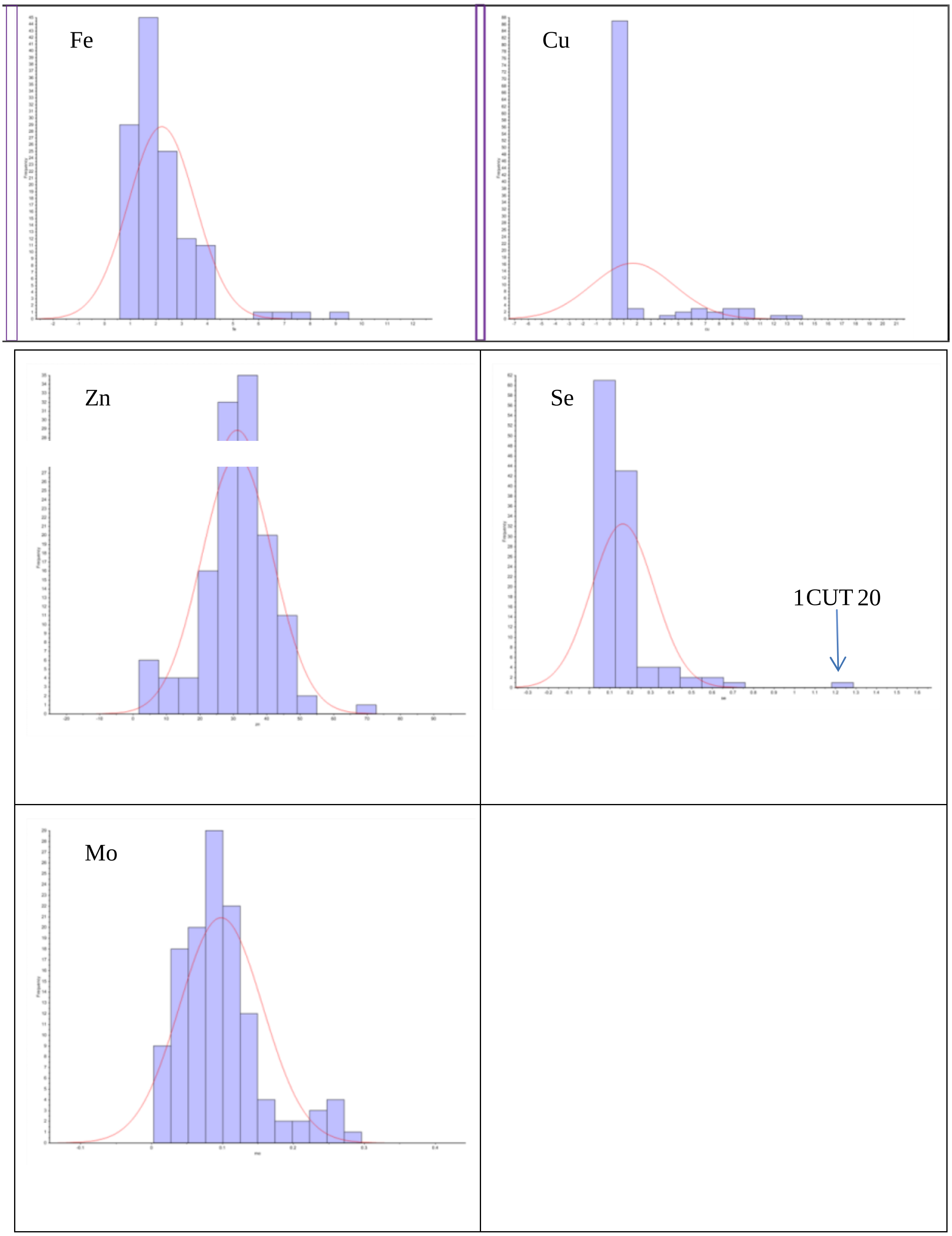


Figure 4. Histograms and overlaid normal distribution curves for elemental and isotope data



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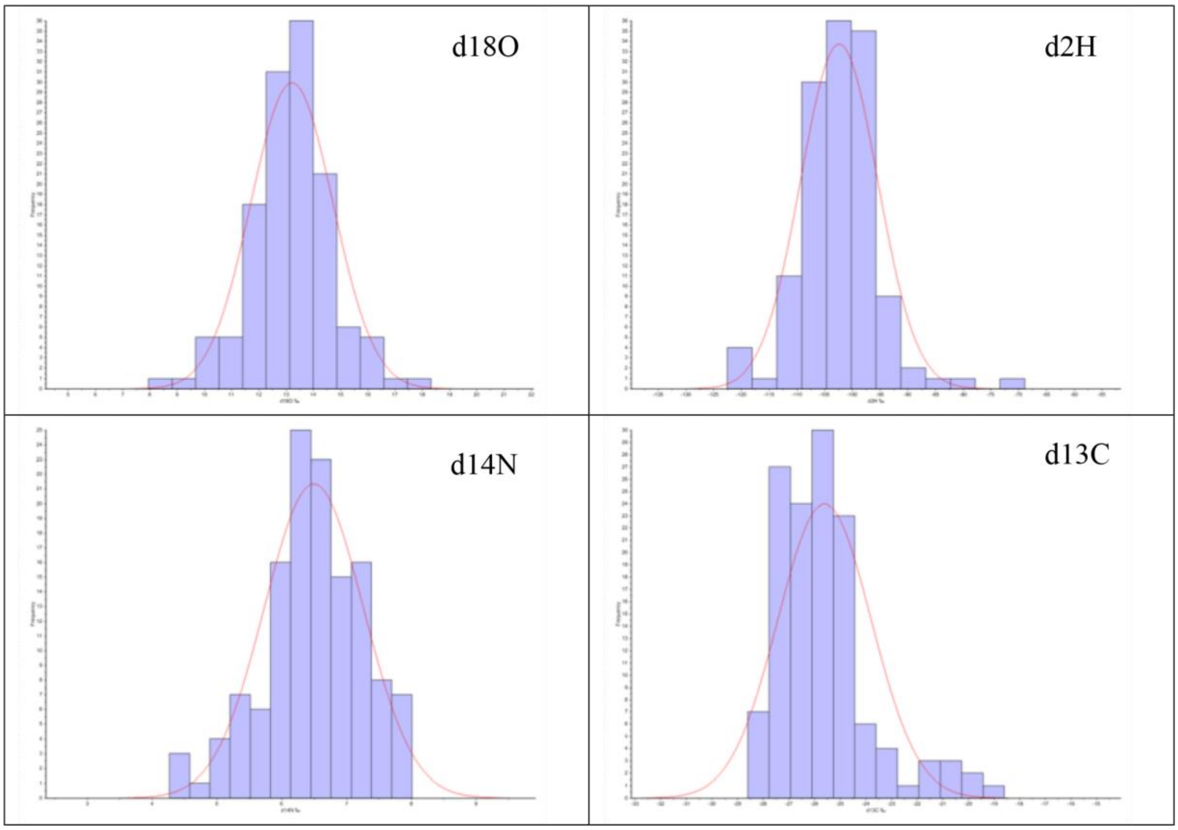

Figure 4 continued

In the case of the majority of the elements, a distribution reasonably close to normal was observed, especially given the relatively small sample size in this work. This inference was also supported by the outcome of the Kolmogorov-Smirnov test for normality on each analyte (Figure 5).

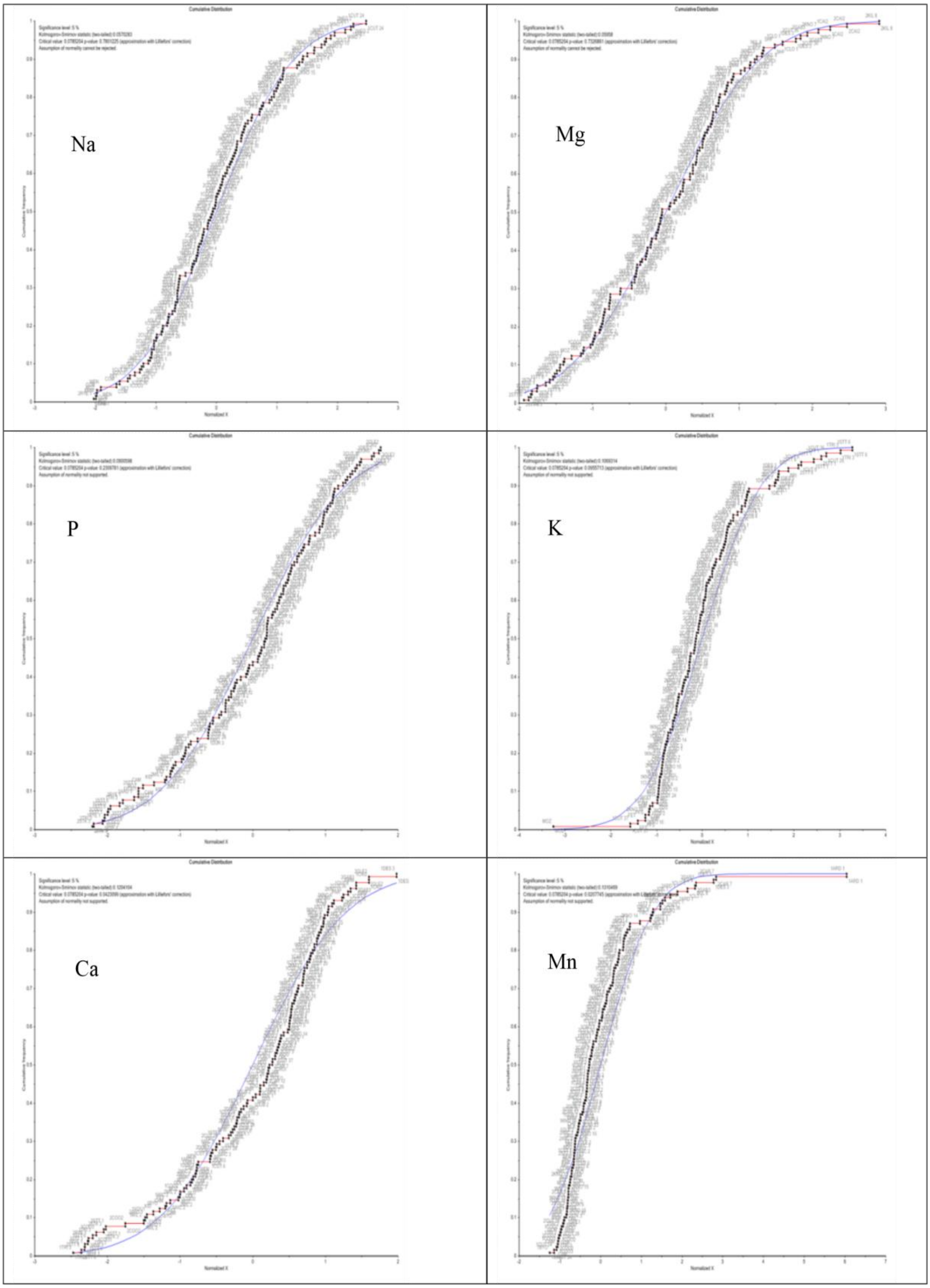

Figure 5: Kolmogorov-Smirnov normality test outputs

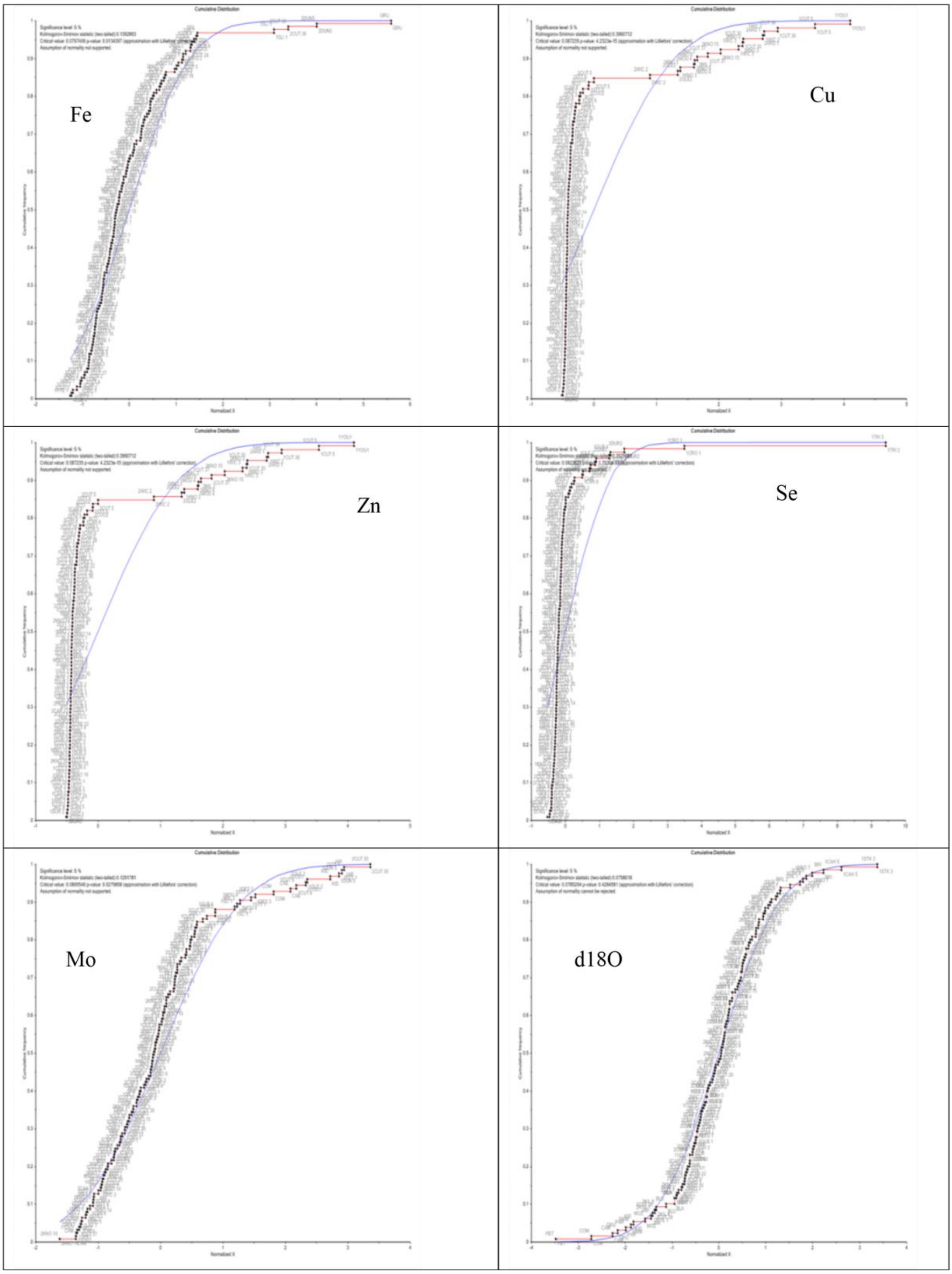

Figure 5 continued

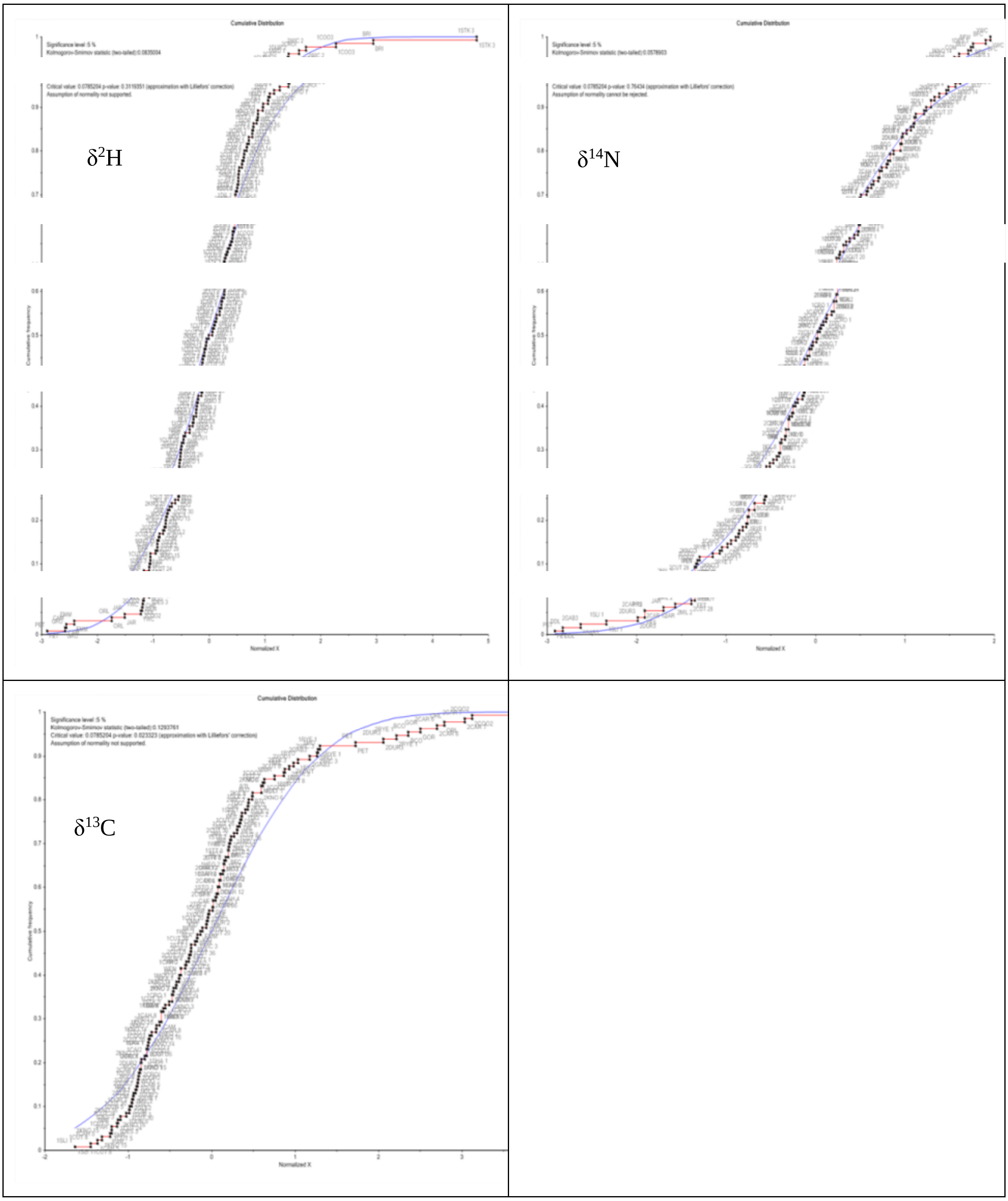

Figure 5 continued

For a Kolmogorov-Smirnov normality test, the actual sample value cumulative distribution function (stepped red curve) is plotted along with the expected cumulative distribution function (blue smooth curve). If the two curves significantly depart from each other over part of the curve, this is an indication that the sample distribution is non-normal. If the two curves follow each other closely, then this is an indication that the sample distribution is normal. However, Figure 4 shows that, for the elements Mn, Fe and Se, some concentration data values were quite far removed from the overall sample set. The distribution for Cu is also skewed but, in the case of this particular element, its concentration will be significantly affected by the extent to which copper equipment is used during the cheese-making process. Values for Fe may also reflect process techniques or conditions. Regarding Mn and Se, the suspicion is that these results are outliers which should be deleted from the analytical dataset before multivariate data analysis. It is believed that the main problem encountered related to the physical sample with sample 1CUT20 in particular being very inhomogeneous. Histograms for the isotopic data are shown in Figure 4. In general, these display close to normal distribution curves with no outlying data points.

Results for the natural isotope ratios of each of the cheeses are shown in Appendix 3. These data were analysed in each geographic origin group; box plot whisker graphs of the data are shown in Figure 3 along with the statistical data (Appendix 5). For C and N isotopes, there is no significant difference from the mean of the cheeses from each region (P-values of 0.0819 and 0.3707). C isotopes are influenced mainly by the diet of the animals from which the milk is produced. $\delta^{13}C$ is related to the content of corn in the animal diet, a C4 plant with higher ^{13}C content ($\delta^{13}C$: −10 to −14‰) than C3 plants ($\delta^{13}C$: −22 to −36‰) such as wheat (Camin *et al.*, 2012). Our results indicate that the animals were fed on very similar diets across the regions.

There were trends in the data for hydrogen and oxygen isotopes. According to Camin *et al.*, (2012), it has been shown experimentally that around 30% of the H in animal protein is derived from local drinking water. Isotope composition of this source is linked to latitude, elevation, distance from the evaporation source, temperature, and the amount of precipitation. Bontempo *et al.*, (2012) cited this finding for isotopic composition (H & O) and also indicated that differences in O isotope ratios may be due to $\delta^{18}O$ being lower at higher altitude as there is lighter water vapour.

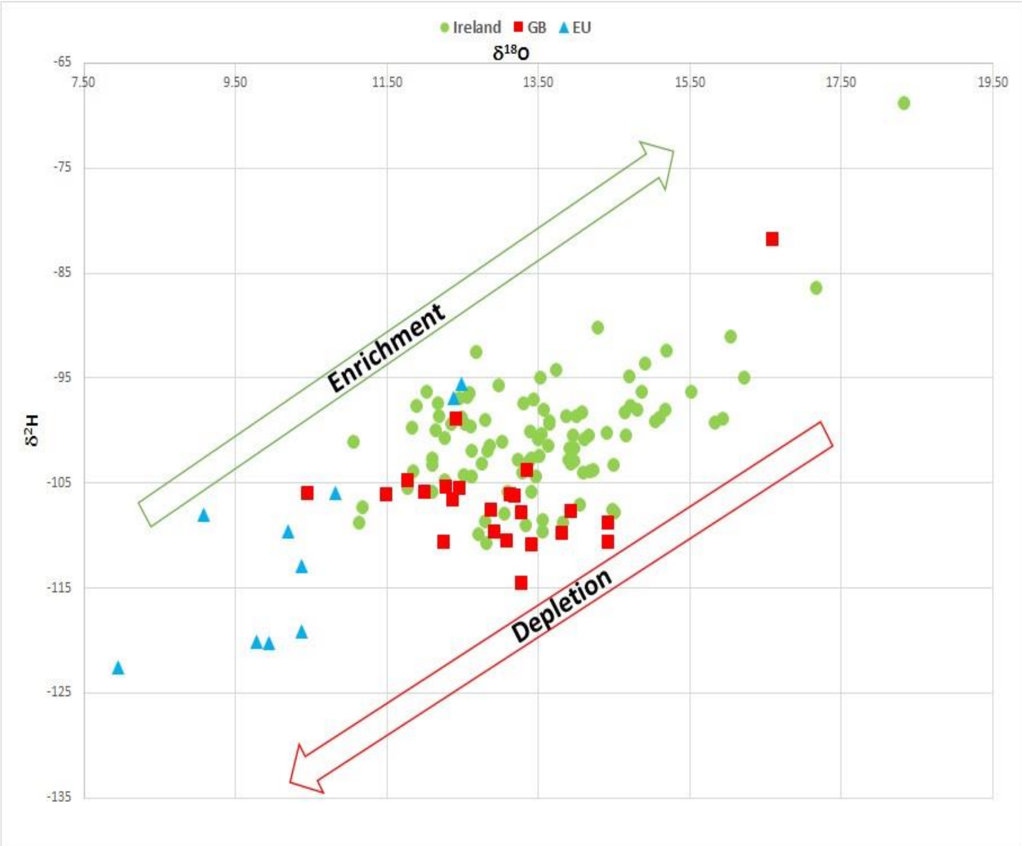

Figure 6. Scatter plot of δ^2H versus δ^{18}O values in tested cheeses

There are quite a number of variables that can influence isotopic ratios in agricultural products and so it was quite interesting to see some trends develop between cheeses from the IoI, GB and mainland Europe. The box plot whisker graphs in Figure 3 show a trend for more positive ‰ values for the cheeses from the IoI. It can be seen from the mean ± std. dev. for the δ 2H data in Appendix 6, that the cheeses from the IoI are more enriched (-100.6 ± 5.6‰) than those from GB (-106.3 ± 6.2‰) or mainland Europe (-111.1 ± 9.7‰). A one-way ANOVA test indicated a significant difference between regional means based on the P-value of <0.0001. Similar observations were found for the δ 18O data, with the mean value for IoI cheeses (13.55 ± 1.27‰) showing more enrichment compared to those from GB (13 ± 1.2‰) or mainland Europe (10.4 ± 1.4‰). In this case, a one-way ANOVA indicated a significant difference between the means based on the P-value of <0.0001. However it can be observed that, from the values for the standard deviation and the box plot whisker graphs, there is an overlap between samples from the different geographic regions. A scatter plot of the δ 2H versus δ 18O data (Figure 6) reveals a general trend of increasing enrichment going from the mainland Europe to GB to the IoI, although considerable overlap is also apparent.

Multivariate analysis of mean results

Elemental data alone

The first step in the multivariate analysis was the visualisation of the sample locations in multivariate space following the application of principal component analysis. This procedure has the potential to detect any underlying clustering of the samples that would not be detectable using univariate methods. An overview of the principal component (PC) score plots is shown in Figure 7.

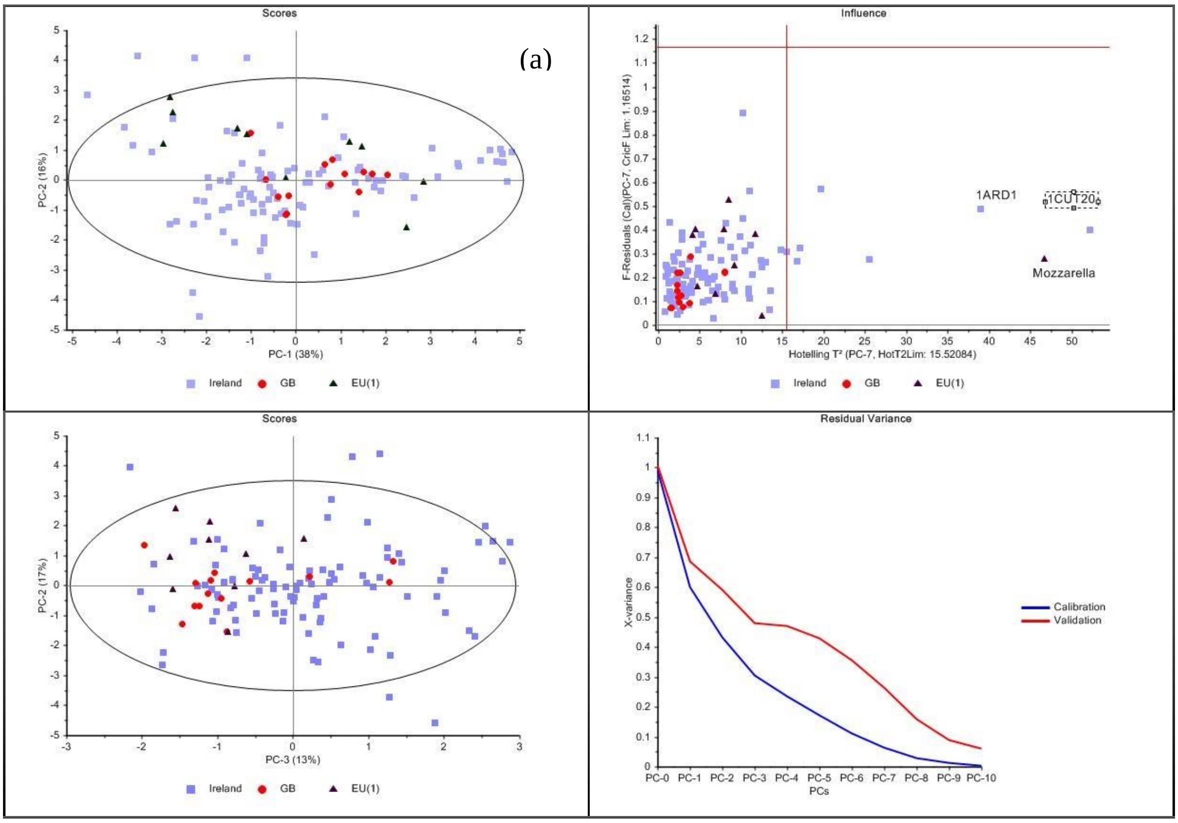

Figure 7. Overview of principal component scores plots for elemental data only; (a) scores plot on PC1 and PC2; (b) leverage and F-residual plot for 7 PC model; (c) scores plot on PC1 and PC2 for attenuated sample set; (d) residual variance plot for attenuated sample set.

It can be seen from Figure 7(a) that a number of samples are found outside the Hotelling's T-squared ellipse (95% confidence limits) but none are far enough outside in the PC1-PC2 plot to be considered as outliers in these dimensions. However, it can be seen in Figure 7(b) that three samples (1ARD1, 1CUT20 and Mozzarella) exhibit high leverage values that are some distance from their nearest neighbours in Figure 7(b). In PC4, the Mozzarella sample is clearly very different from the other cheese samples while the replicate elemental analyses for samples 1ARD1 and 1CUT20 were quite different due to sampling problems. For this reason, these three samples were removed from the sample set; the PC1 vs PC2 score plot for this attenuated sample set is shown in Figure 7(c); no significant outliers were apparent in a visual examination of score plots involving PCs 1-10. In general, the data exhibit classic decomposition behaviour with the sample set residual variance reducing incrementally with increasing PCs (Figure 7(d)).

It is also apparent from a visual examination of the PC scores plots that little or no clustering of samples on the basis of geographic origin can be detected. The variability in elemental composition of the IoI artisanal cheeses creates a multivariate space in which most, if not all, of the GB and mainland European cheeses are found. In this regard it is worth noting that in a key article describing similar work in Italy, Camin et al. (2012) reported strontium (Sr), rhenium (Re), uranium (U), bismuth (bi), nickel (Ni), gallium (Ga) and lithium (Li) to be significant in addition to copper (Cu), molybdenum (Mo), sodium (Na), iron (Fe), manganese (Mn) and selenium (Se) which were measured in this study.

Discriminant modelling

A summary of the performance of the best two-class models developed using elemental data alone is shown in Appendix 6. In the first case, models to discriminate between IoI and GB cheeses failed completely in the validation step; comparable models to discriminate between IoI and mainland European cheeses produced quite a high level of correct classification for IoI cheeses (90.5%) but only 50% correct classification in a very small set of mainland European cheeses. For the model to distinguish IoI cheeses from a combined GB and mainland European sample set, model performance was quite good with 83.8 and 66.7% correct classification rates, respectively. An alternative modelling strategy involving IoI and GB cheeses as a single category produced high levels of correct classification in the case of both groups i.e. 97.3% for the combined IoI & GB cheeses and 80% for cheese samples from mainland Europe.

Class-modelling

Using elemental compositional data, the sensitivity of IoI cheese classification was 0.90, with 43 out of 48 prediction samples correctly classified (Appendix 8). Regarding the other class, however, specificities were very low with values of 0, 0.1 and 0.03 for GB, European and GB+Europe cheeses respectively. Using the IoI+GB versus mainland Europe modelling, sensitivity and specificity values of 0.74 and 0.80 respectively were obtained.

Isotopic data alone

Discriminant modelling

In general, the correct classification of samples in each of the two-class systems was an improvement over the results obtained using elemental analysis. In an examination of the prediction set results (the best indicator of long term performance), it can be seen that the highest correct classification for IoI samples was obtained in the IoI vs mainland European samples model, with 81 out of 87 (93.1%) being correctly identified. However, it is not possible to be confident about the real accuracy of mainland European sample identification given the very small number of samples available. In the case of IoI+GB cheeses versus mainland European samples, prediction results revealed correct classification rates of 96.4 and 80% for these groups respectively. Using isotope data only, the most accurate model was therefore that discriminating between IoI+GB and mainland European cheeses but, given the low sample number of cheeses from the latter source, and the small number of samples which could be used in model development, the long term accuracy of this model is questionable.

Class-modelling

Model sensitivity in this case was lower than with the elements only (see above) at 0.79. However, specificity values were higher ranging from 0.35 to 0.70 (Appendix 8). The maximum specificity value (0.70) was achieved in the case of the mainland European cheese prediction set, an observation that agrees well with the previously noted relative enrichment of δ 2H and δ 18O in cheese samples shown in Figure 6. Using the combined GB+Europe class, a specificity value of 0.45 was obtained reflecting the poor performance in identifying GB samples as different from those of IoI provenance. Using the IoI+GB class, sensitivity and specificity values of 0.96 and 0.6 were achieved. While this level of model sensitivity is encouraging, the specificity casts doubt on the long term accuracy of the model for cheeses from mainland Europe. It is worth noting that Camin et al. (2012) reported δ 34S (not measured in this study) as a significant isotope in the confirmation of Grana Padano cheese provenance.

Combined elemental and isotopic data

Discriminant modelling

Accuracy values for entire data set were approximately the same as those for either elemental or isotopic data type alone. Once again, the IoI versus mainland European sample discrimination appeared best although the caveat about the small number of European samples remains. Prediction of geographic origin using the IoI+GB vs mainland Europe classes produced data of marginally higher accuracy than the IoI vs mainland Europe classification.

Class-modelling

Using this combined data set, the sensitivity value obtained on the prediction sample set was the highest at 0.96 i.e., 46 out of 48 samples from the IoI were correctly classified (Appendix 8). However, specificity results were poorer than those obtained using isotope data only, probably reflecting the very poor performance of the elemental analyses. In this case, their incorporation degraded the isotope model. This poor specificity means a very high rate of false positive identifications i.e., non-IoI samples being incorrectly identified as from the IoI. In the case of the IoI+GB model, specificity and sensitivity values of 0.82 and 0.2 reveal a medium level of accuracy in the classification of IoI and GB cheeses but a low classification accuracy of cheese samples from mainland Europe.

Project Conclusions

- The analytical strategy pursued has generated a considerable quantity of baseline data on the elemental and isotopic composition of IoI artisanal cheese. In this regard, an extensive databank of IoI artisanal cheese samples has been developed.
- Trends in some of the data, especially the isotope data, suggest the possibility of segregation of IoI from mainland European, if not GB, cheeses.
- On the basis of the analytical data produced and the multivariate data analysis methods investigated, it was not possible to unambiguously confirm the geographic provenance of a cheese which claimed to be from the IoI as being produced on the IoI. However, models developed may be suitable for use as quality control tools for IoI producers who wish to indicate the geographic provenance of their products.
- Examination of IoI+GB versus mainland European cheeses produced results which suggested high correct classification rates for IoI+GB cheeses as a group, and moderately high values for cheeses from mainland Europe. However, these results must be treated with caution in view of (a) the very small number of cheese samples from mainland Europe and (b) the use of only a small number of samples in each cheese category that may be used (for statistical reasons) in model development.
- The results indicate that further investigations should focus on a narrower range of cheese types (as is the case in the Italian examples cited here).
- The results provide a firm basis for a more conclusive follow-on study that would cover a greater geographic region and time span. In addition, the analysis of a greater number of elements (particularly transition elements and δ 34S), and the application of alternative multivariate data analysis procedures should overcome some of the obstacles encountered here and further the development of robust provenance assurance models.

Added value & benefits of research

This research project provided an excellent opportunity for a North-South technical collaboration between Teagasc-Ashtown Food Research Centre in Dublin and the Institute for Global Food Security in Queen's University Belfast. It has contributed enormously to capacity building in terms of analytical expertise on the island of Ireland by facilitating the introduction of an IRMS capability in QUB and extending the analytical capability of the ICP-MS facility within Teagasc. Interaction between the Teagasc ICP-MS analyst and the Italian laboratory operating the Grana Padano cheese authentication scheme in Italy is ongoing and has benefitted both the Teagasc and QUB analytical operations. Furthermore, the project involved all of the key stakeholders in the artisan cheese food chain on the island of Ireland, including both food safety regulatory agencies, and has resulted in a database of the artisan cheese producers and samples of their products.

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Appendix 1

Project Management Board

- Prof Gerard Downey, Teagasc (Chairperson)
- Bernard Corrigan, Teagasc
- Prof Christopher Elliott, Institute of Global Food Security, QUB
- Dr Simon Haughey, Institute of Global Food Security, QUB
- Dr Rachael Hill, Institute of Global Food Security, QUB
- Dr James McIntosh, *safe*food
- Federica Camin, FEM-IASMA
- Marion Roeleveld, Cáis - The Association of Irish Farmhouse Cheesemakers
- Elisabeth Ryan, Sheridan's/Cáis
- Eddie O'Neill, Teagasc
- Nick Finnerty, Department of Agriculture, Food and the Marine
- Dermott Jewell, Consumers Association of Ireland
- Dr Patrick O'Mahony, Food Safety Authority of Ireland
- Mervyn Briggs, Food Standards Agency Northern Ireland

Appendix 2

Sample handling and analysis

Chilled IoI cheeses were delivered to the Teagasc-Ashtown Food Research Centre where they were logged, coded, sub-sampled and placed in storage at -20°C. Non-IoI cheeses were treated similarly at the Institute for Global Food Security in QUB. Sub-samples of all cheeses were delivered frozen to laboratories in the Teagasc-Moorepark Food Research Centre and QUB prior to analyses. A total of 131 cheese samples was analysed; 98 IoI (88 RoI and 10 NI), 23 GB and 10 from mainland Europe. Samples of all cheeses remain in frozen storage at Teagasc-Ashtown.

Inductively coupled plasma mass spectrometry

Care was taken to prepare and store all samples and utensils in as clean a manner as possible. All plastic ware and any other materials which were to come into contact with the ICP-MS samples or standards was cleaned first in 5% nitric acid and then in MQ water to prevent contamination. Cheese samples were stored at -200°C before analysis. Upon defrosting, approximately 50g of each cheese was prepared by grating using an electric cheese grater (Moulinex type 243200, Ecully, France). After homogenising the grated sample, 0.5g of cheese was weighed out in duplicate and placed into closed PTFE digestion vessels to which were added 7ml of nitric acid (69%, trace SELECT, Fluka) and 2ml of Ultrapure water (Millipore, Bedford MA). Samples were then digested and mineralised in a MARS Microwave oven (Mars Express, CEM). Digests of the cheese samples were then analysed for Ca, Na, Mg, P, K, Mn, Cu, Se , Fe, Mo and Zn using an Agilent 7700x ICP-MS with an Octopole Reaction system. Argon was used as the carrier gas and helium as the collision gas to minimise polyatomic interferences.

The accuracy of the instrument was checked for each mineral in each batch by measuring recovery against a certified standard (ERM BD 151, skim milk powder, Institute for reference materials and Measurements, Geel, Belgium). These recoveries were all within 80-120% of the known values with the between majority lying between 90-100 %. Analytical results for batches for which recovery values lay outside this reference range were not used. The system was also checked using an online internal standard mix solution containing 10 mg/L of Bi, Ge, Ln, Li, Sc, Tb and Y (Agilent Technologies). Method precision was assured by analysing the same cheese (Cheshire white) ten times; RSD% of the repeated samples was between 3.87% (Mo) to 5.644 %(Cu) for all of the elements analysed with the exception of Iron, which gave an RSD of 11.83%. The limit of detection (LOD) for each element was calculated by digesting and analysing nine blank samples (MQ water) and adding two times the standard deviation of the blank to its mean.

Isotope-ratio mass spectrometry

Preparation of cheese for IRMS analysis

Preparation of defatted cheese caseins for IRMS analysis was based on the protocol described by Camin et al. (2012). Frozen cheeses were finely grated and freeze-dried overnight (model Christ Alpha 1-4 LD plus). Dried cheese (4g) was placed in a 50 ml disposable centrifuge tube (VWR catalogue number 525-0463) and extracted three times with a 2:1 mixture (30ml) of petrol ether (Fisher):diethyl ether (Sigma Aldrich), followed by water washing of the pellets twice (using 30 ml of MilliQ water from a Millipore Q-Pod). The first two extractions were performed using an Ultra-turrax homogeniser (T-25 basic, Stauffen, Germany; 13,500 rpm for 3 mins) and all subsequent steps using a vortexer (VWR DVX-2500 Multi-tube Vortexer) at 2500 rpm for 3 mins. After each extraction the casein pellet was recovered by centrifugation (Sorvall Legend RT, 4100 rpm for 5 mins). The final residue was lyophilised, hand ground using a glass pestle and mortar, and stored at room temperature prior to analysis.

Measurement of Carbon and Nitrogen Isotopes

The ratios of 13C/12C and 15N/14N were measured simultaneously using a Sercon 20-22 Stable Isotope Ratio Mass Spectrometer (Sercon, UK) following combustion in a Europa EA-GSL Sample Preparation System (Sercon, UK). Three replicates of 0.7 mg of casein were weighed into tin capsules (Sercon, UK, 4 x 6mm), and the results of the replicates averaged. The oxidation oven was set at 1000 °C, the reduction oven set at 890 °C, the second reduction chamber was maintained at 600 °C, and the GC at 60 °C. Helium carrier gas flow rate was ~100 ml/min. δ 13C and δ 15N values for each cheese were calculated by calibration against a previously calibrated in-house standard (IHS) casein, the values of which were assigned and drift corrected in a single point calibration using the Sercon Callisto software (v 10.0.72). Each set of three cheese replicates (9 samples) bracketed by triplets of the IHS also contained a single QC for nitrogen and carbon results (USGS 40; USGS Isotope Laboratory, Reston, VA 20192). Data for the entire bracket was rejected and the analysis repeated if the QC deviated more than +/- 0.3‰ from the expected value, and for individual cheeses if the triplicates had a standard deviation of >0.3‰ for δ 13C and δ 15N.

The IHS casein was defatted using the same procedure above and calibrated against reference standards L-glutamic acid (USGS 40) and sucrose (IAEA CH-6; IAEA, Vienna, Austria) for δ 13C and against USGS 40 for δ 15N, by running four sets of six samples of the IHS against the calibration reference, over three separate runs (72 replicates in total). Within each of the four sets several QCs were included (both IAEA CH-6 and USGS 40 for δ 13C and USGS 40 for δ 15N and if the expected value of any QC deviated by more than +/- 0.4‰, that set of six replicates was discarded. The δ 13C IHS calibration was based on 60 replicates using USGS 40, 66 replicates using IAEA CH-6, and the δ 15N calibration was based on 60 replicates (out of the total of 72).

Measurement of Hydrogen and Oxygen Isotopes

Hydrogen and oxygen isotope ratio analyses were performed separately using a Sercon 20-22 Stable Isotope Ratio Mass Spectrometer (Sercon, UK) following pyrolysis in a Sercon High Temperature Elemental Analyser fitted with a glassy carbon tube. The pyrolysis oven was set at 1350°C, the GC at 60°C and the helium carrier gas flow rate was ~60 ml/min. Triplicates of ~0.5 mg of casein were weighed into silver capsules (Sercon, UK, 4 x 6mm), and the results averaged. The compacted capsules of cheese and IHS caseins were dried under vacuum over self-indicating phosphorous pentoxide (Sigma-Aldrich) for at least five days prior to analysis, and were loaded into a helium purged autosampler. Each batch run included six samples at the start to condition the detector and, between batches, the oven was cooled and the glassy carbon chips refreshed.

Batch structure was similar to that for nitrogen/carbon analysis; triplicates of a reference standard were run before and after nine samples (three replicates of three cheeses) and included in each bracket was a triplet of IHS casein and a QC. After the run, the reference samples' values were assigned and drift corrected in a single point calibration using the Sercon Callisto software (v 10.0.72). For δ 2H analysis the reference standard was IAEA CH-7 (polyethylene; IAEA, Vienna, Austria) and the QC was IAEA NBS 22 (oil, USGS, Isotope Laboratory, Reston, VA 20192). Samples were repeated if the QC within the bracket was more than +/- 3.0‰ from the expected value, or if the triplets of any cheese had a standard deviation of >3.0‰. For δ 18O analysis, the reference standard was IAEA 601 and the QC was IAEA 602 (both benzoic acids; IAEA, Vienna, Austria). Samples were repeated if the QC within the bracket was more than +/- 1.1‰ from the expected value and if the triplets of any cheese had a standard deviation of >0.8‰.

The IHS triplet was included in each bracket to enable a more rigorous two-point calibration to be constructed; this is now accepted as the norm for δ 2H determination. However this required further work in calibration of the IHS against reference materials, including determination of the non-exchangeable δ 2H value of the IHS casein via an enriched/depleted water incubation experiment as described by Meier Augenstein et al. (2014).

Analytical uncertainty was calculated from the standard deviation across all runs of the QC standards, and was 0.2‰ for δ 15N based on USGS 40, 0.13‰ for δ 13C (USGS 40), 0.52‰ for δ 18O (based on IAEA 602) and 1.3‰ for δ 2H based on NBS 22). Isotope data are reported in conventional δ -notation in units of per mil (‰) with respect to a standard. For example Nitrogen isotope data are reported with respect to atmospheric nitrogen (air) according to the equation below:

$$\delta^{15}N_{sample}(‰) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right) \times 1000$$

where R is $\frac{abundance\ of\ heavy\ isotope}{abundance\ of\ light\ isotope}$

Data analysis

Univariate data analysis

Data were assembled in Excel and imported into the Unscrambler X (vs 10.3; Camo, Norway) for analysis. Standard calculations describing the mean, dispersions, range etc., were performed on the entire data collection. The Kolmogorov-Smirnov normality test was applied to support visual assessments of normality for the distributions of each element and isotope based on histogram creation.

Multivariate data analysis

Initial investigations of dataset structure were performed by principal components analysis (PCA). Given the different scales and ranges of the variables, all data were normalised (values for each variable were divided by the standard deviation of the same variable over the entire sample set) before the application of any multivariate procedure. Discriminant analysis was performed by discriminant partial least squares (D-PLS) regression using dummy values of 1 and 2 for the IoI and other non-IoI respectively. A cut-off value of 1.5 was selected as the determinant of prediction sample classification. Models with the optimum number of D-PLS loadings were selected on the basis of the number which produced the first local minimum in the prediction error as a function of the number of latent variables incorporated. Models were developed and validated using leave-one-out cross-validation owing to the limited number of samples available. It is a feature of this type of validation strategy that the models produced may be slightly optimistic. A series of two-class models was investigated; (1) IoI vs GB; (2) IoI vs mainland Europe, and (3) IoI versus GB + mainland Europe. In each case, models were developed using (a) elemental data only; (b) isotope data only, and (c) all analyte data. The same samples were used in each class model. During model development, equal numbers of samples from each class were used in the calibration exercise given the sensitivity of this step to unbalanced data sets; this was not possible for cheeses from mainland Europe given the small number analysed (10). Soft independent modelling of class analogy (SIMCA) was the class-modelling technique used to try to address the classification issue studied in this project. SIMCA models describing cheese from the IoI were developed using the aforementioned three data categories (a), (b) and (c). In this case, the SIMCA models were produced using 50% of the IoI samples; model validation was performed on the balance of the entire sample set. All results were obtained using $p=0.05$.

Appendix 3

Mean results of all cheese analyses (m= missing value)

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	$\delta^{18}O$ ‰	$\delta^{2}H$ ‰	$\delta^{14}N$ ‰	$\delta^{13}C$ ‰
1	6641.02	185.54	3922.90	953.39	4095.17	1.37	1.81	0.22	25.25	0.10	0.03	12.67	-92.47	6.71	-27.15
2	4795.17	228.69	3659.51	808.29	5210.03	0.34	2.19	0.34	33.24	0.13	0.11	12.82	-101.88	6.68	-24.00
3	5406.97	175.77	3176.82	744.47	3891.58	0.16	1.73	0.44	25.55	0.14	0.09	11.77	-105.51	5.98	-24.23
4	5681.93	160.22	2994.30	934.50	2926.23	0.10	1.10	0.28	20.47	0.16	0.13	12.62	-104.39	6.27	-23.72
5	6292.92	278.32	5333.32	838.31	7778.80	0.39	3.22	m	34.66	0.09	0.11	17.17	-101.04	5.70	-28.11
6	7107.04	318.02	5021.84	1092.30	7070.31	0.26	1.51	0.23	32.27	m	0.06	13.02	-98.87	7.36	-25.52
7	6007.53	255.09	4618.25	804.95	6808.85	0.28	2.54	m	31.26	0.09	0.09	15.93	-109.02	6.59	-26.75
8	6534.03	474.22	6517.58	925.82	9485.12	0.37	m	0.82	37.91	0.34	0.05	13.34	-100.46	6.69	-27.07
9	8602.14	287.33	4078.52	847.96	6299.16	0.28	2.66	m	21.94	0.23	m	14.65	-98.27	6.11	-25.44
10	8632.78	333.32	5033.84	1011.71	7551.09	0.25	2.23	m	29.27	0.15	0.06	14.07	-97.98	6.30	-25.44
11	5809.37	312.86	4642.23	962.11	7415.96	0.26	2.56	m	28.19	0.18	m	14.80	-100.84	6.47	-26.28
12	6967.68	413.88	5251.38	844.50	7848.63	0.69	m	1.02	33.35	0.19	0.03	14.10	-97.96	7.05	-26.98
13	6403.84	160.93	3708.42	1048.54	3566.76	0.14	0.91	m	28.62	0.15	0.06	13.57	-99.09	5.49	-24.45
14	3500.69	95.51	1941.00	1001.39	947.97	0.13	0.62	0.23	14.40	0.12	0.08	15.05	-86.42	5.84	-26.99
15	7452.28	319.75	5204.31	749.88	6940.80	0.30	2.04	0.65	28.55	0.46	0.04	15.18	-98.05	6.64	-26.54
16	7902.40	303.11	5755.30	879.56	7639.34	0.38	2.02	0.32	35.84	1.29	0.10	13.93	-103.09	6.75	-25.72
17	11337.69	182.61	4577.17	708.68	5355.86	0.12	0.87	0.33	36.92	m	0.06	13.56	-109.67	6.69	-27.66
18	4645.88	371.98	5520.35	1621.59	6267.22	0.23	1.34	0.29	20.43	m	0.05	13.09	-105.79	6.43	-25.05
19	8160.05	305.74	4991.01	882.81	7461.31	0.17	1.60	0.31	37.34	0.14	0.10	14.04	-106.99	6.31	-27.41
20	3576.40	344.33	4830.07	953.84	7431.25	0.37	2.07	0.20	30.70	0.18	0.10	13.54	-100.33	6.27	-26.05

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
21	7270.65	221.77	4323.04	554.64	6550.66	0.41	2.18	0.36	28.66	0.17	0.08	12.63	-101.97	7.49	-26.62
22	7046.20	242.00	4622.54	1022.75	7010.09	0.25	1.32	0.26	32.07	m	0.07	14.18	-103.89	7.52	-27.80
23	4182.32	299.37	3645.25	1361.16	4506.09	0.20	1.10	0.54	23.32	0.10	0.02	12.14	-99.92	6.54	-25.04
24	9630.99	365.72	5636.40	1485.60	8909.04	0.31	3.70	0.57	36.30	0.11	0.13	14.50	-103.22	5.93	-28.25
25	7854.86	430.72	6437.96	1364.06	10345.03	0.80	2.83	8.92	40.29	0.12	0.11	12.71	-109.81	7.84	-27.68
26	3902.14	271.07	5136.93	1313.88	6083.38	0.61	1.63	10.59	27.97	m	0.23	15.83	-99.17	7.35	-26.71
27	5135.59	259.43	5731.02	966.00	8064.46	0.30	1.39	7.19	37.24	0.15	0.19	13.50	-100.80	7.25	-27.47
28	5823.73	233.00	4086.66	867.23	5346.01	0.39	2.37	m	26.92	0.14	m	15.20	-92.37	7.34	-25.68
29	5049.20	221.76	3630.80	868.09	4072.85	0.32	2.57	m	21.28	0.09	0.08	14.71	-97.67	6.41	-27.40
30	4658.41	258.12	5915.26	1053.09	7667.34	0.21	1.19	12.39	43.28	m	0.27	12.80	-108.65	6.41	-24.81
31	5160.57	276.14	4470.43	907.57	5846.86	0.23	1.66	0.40	33.48	0.15	0.09	13.87	-98.61	7.49	-27.25
32	6769.99	312.47	4901.83	968.94	7223.63	0.28	3.19	m	30.92	0.11	0.08	13.74	-94.23	7.62	-27.22
33	7596.08	296.03	5579.24	735.71	7920.77	0.26	1.47	0.44	38.07	0.17	0.24	13.64	-101.39	6.69	-25.36
34	9292.94	298.99	4636.73	1215.09	7232.97	0.32	m	0.47	38.46	0.19	0.12	13.89	-102.82	6.07	-25.57
35	5192.43	389.83	5571.33	714.64	8221.03	0.72	4.03	0.65	28.08	0.42	0.06	14.10	-103.95	6.19	-27.23
36	7092.12	322.87	5083.80	760.26	7723.02	0.35	1.41	0.24	36.22	0.13	0.15	13.98	-102.92	7.70	-26.96
37	10182.33	282.39	5829.17	712.59	8094.34	0.17	1.12	0.30	37.75	m	0.13	13.83	-108.80	5.82	-28.01
38	7366.07	304.52	4900.44	731.57	7253.97	0.22	1.30	0.15	23.62	m	0.09	14.86	-96.30	6.27	-26.82
39	6162.15	381.42	5161.98	966.98	7045.26	0.34	1.90	0.32	26.12	0.47	0.03	12.09	-103.29	7.05	-25.41
40	8455.93	325.48	5595.19	819.01	8387.35	0.41	1.96	0.38	34.02	0.16	0.11	13.46	-104.31	7.07	-26.71
41	10657.59	332.24	4975.03	1003.47	6192.82	0.34	m	0.51	23.59	0.60	0.04	16.22	-95.01	6.35	-26.47
42	7958.76	333.28	5042.00	1019.06	7279.47	0.26	m	0.36	34.17	0.18	0.13	13.04	-107.91	5.68	-27.03
43	4016.65	117.95	3299.67	1182.51	2432.30	0.18	0.88	m	19.01	0.06	0.09	15.51	-96.27	6.33	-27.31

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
44	5754.35	269.48	6069.71	954.38	8065.10	0.44	1.69	0.55	40.90	m	0.13	13.37	-102.94	6.28	-26.37
45	5631.83	101.87	1845.58	1180.81	576.55	0.27	0.78	0.54	1.80	0.11	0.02	11.85	-103.86	5.92	-23.24
46	7243.13	299.58	4800.80	1115.66	7180.34	0.54	1.61	0.49	33.02	0.08	0.17	14.22	-103.74	7.14	-27.03
47	9876.65	334.41	5737.21	875.95	8183.80	0.37	2.16	0.31	39.63	0.14	0.17	13.51	-102.39	4.71	-28.60
48	10846.62	194.17	3209.21	968.49	4093.04	0.17	1.95	0.39	26.49	0.14	0.09	13.97	-101.62	7.35	-24.95
49	4856.05	207.56	4062.33	661.88	4917.43	0.26	0.60	5.86	27.55	0.09	0.07	12.80	-99.01	7.64	-25.22
50	4290.45	305.04	5350.46	784.93	7543.36	0.28	1.12	6.56	38.80	0.10	0.17	12.51	-104.26	6.77	-25.50
51	5803.15	288.42	3923.60	1082.62	5558.52	0.32	6.25	m	30.83	0.07	m	18.32	-68.86	6.45	-26.66
52	4241.39	127.12	1873.82	1555.13	591.80	0.54	1.11	0.41	7.99	m	0.02	12.56	-96.81	6.88	-24.48
53	4969.40	112.89	1926.81	1764.87	677.71	0.34	1.06	0.38	7.39	m	0.03	13.65	-99.09	7.11	-25.30
54	5617.42	91.13	2129.77	1701.18	228.23	0.14	0.67	0.66	5.67	m	0.05	12.45	-96.96	7.14	-25.34
55	6370.90	193.38	2986.42	834.07	3266.11	0.39	1.87	m	19.70	0.10	0.09	14.00	-98.63	6.24	-25.24
56	5769.31	235.18	3334.21	806.03	4691.16	0.27	2.26	m	21.50	0.13	m	13.92	-101.74	5.88	-25.93
57	6604.50	135.27	2446.02	1078.70	2524.30	0.26	1.53	6.52	43.22	0.25	0.18	12.26	-104.75	5.48	-25.71
58	8430.02	124.25	2376.07	874.51	2473.19	0.65	2.56	0.36	14.43	0.10	0.03	12.11	-105.89	6.08	-25.12
59	5916.93	329.72	4626.68	786.50	6733.25	0.36	2.82	0.27	34.84	0.11	0.12	12.53	-99.34	6.89	-25.47
60	8317.30	304.42	5508.97	929.28	7776.26	0.32	3.07	0.67	38.84	0.06	0.07	12.85	-101.45	7.04	-27.21
61	7317.96	495.49	5698.79	944.68	7400.01	0.26	1.70	m	29.90	0.27	0.04	14.91	-93.57	7.32	-26.10
62	9249.61	312.20	4761.84	1044.34	7071.09	0.23	2.58	0.98	34.43	0.15	0.02	12.19	-98.68	5.04	-25.41
63	8592.92	283.58	4307.71	1001.40	6083.81	0.18	2.81	0.64	36.78	0.23	0.07	12.59	-96.41	6.66	-20.67
64	7334.81	286.64	4524.70	910.68	6467.47	0.81	1.69	0.74	33.88	0.11	0.04	12.17	-97.40	6.57	-20.07
65	6487.23	122.03	1878.04	916.06	1848.09	0.13	1.48	0.24	24.44	0.08	0.08	12.82	-110.70	6.75	-19.91

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
66	8615.68	387.67	4593.37	726.21	6453.34	0.31	2.72	m	28.45	m	0.06	16.03	-91.03	6.39	-27.15
67	4363.71	194.76	3637.36	953.54	4805.86	0.15	1.31	0.51	29.59	0.15	0.10	11.13	-108.77	5.44	-26.16
68	7227.50	324.80	5558.11	855.12	7884.79	0.30	1.84	m	37.01	0.09	0.13	14.64	-98.22	6.25	-25.18
69	4838.63	436.82	6221.46	936.67	8833.15	0.58	3.97	m	44.83	0.19	0.13	13.41	-102.68	7.12	-27.01
70	10305.37	246.14	4382.47	971.86	6654.39	0.33	3.59	m	34.38	0.11	0.10	13.65	-99.40	6.21	-26.11
71	4370.87	340.83	4017.59	1163.38	5324.80	0.43	3.93	1.44	34.33	0.13	0.02	12.27	-100.73	7.24	-25.57
72	8315.80	350.88	4835.86	1102.99	7160.16	0.25	2.64	0.37	37.27	0.11	0.11	13.29	-103.97	6.84	-24.03
73	5061.73	323.90	5955.25	1341.95	7426.47	0.59	6.65	9.94	46.28	0.02	0.30	14.70	-94.77	7.45	-27.33
74	5138.55	326.02	6399.60	904.87	8335.07	0.33	3.55	8.70	46.65	0.06	0.14	14.40	-100.21	7.23	-26.45
75	6616.39	241.49	3979.92	1061.19	5416.15	0.57	1.51	m	30.83	0.16	0.11	12.98	-95.64	7.23	-27.18
76	5993.21	225.38	3713.12	982.42	4540.39	0.41	0.98	14.10	24.45	0.06	0.05	12.35	-99.33	4.98	-21.85
77	7080.74	445.19	6194.63	1358.82	9082.18	0.73	4.09	9.88	47.67	0.13	0.12	13.41	-105.85	4.49	-23.46
78	3740.27	378.59	6549.73	927.40	9487.73	0.41	7.44	m	72.78	0.10	0.14	13.56	-108.51	6.68	-27.35
79	6925.37	315.52	4988.94	1066.49	6711.03	0.24	2.35	0.55	40.42	0.07	0.05	12.49	-98.69	7.24	-27.27
80	6250.86	308.83	5136.14	1160.65	7046.15	0.37	2.03	0.79	41.51	0.05	0.07	14.17	-100.41	6.06	-26.18
81	10923.09	256.43	5341.47	724.61	6679.69	0.24	1.96	0.56	42.85	0.06	0.15	14.51	-107.78	6.66	-26.81
82	9409.23	225.63	4576.02	1201.27	5270.08	0.40	2.33	m	44.52	0.19	0.10	13.22	-102.79	6.40	-26.39
83	5958.02	536.56	5747.56	1077.15	8052.76	0.53	2.99	m	35.94	0.41	0.05	11.18	-107.28	6.25	-24.80
84	8388.43	342.66	4708.35	879.89	6983.65	0.48	2.81	0.34	37.38	0.12	0.11	13.30	-97.42	6.55	-26.44
85	9077.50	351.92	5985.75	840.43	8686.52	0.19	3.80	0.39	45.61	0.06	0.08	14.48	-107.51	6.14	-27.14
86	9637.58	246.52	4862.20	636.16	7034.09	0.31	2.62	0.67	40.67	0.15	0.09	12.10	-102.71	5.85	-27.60
87	10027.79	349.01	6116.63	1189.03	7135.11	0.37	2.91	m	42.67	0.73	0.03	13.39	-100.06	5.51	-26.47

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
88	8528.46	329.38	5657.42	793.42	7992.95	0.37	3.49	m	37.25	0.08	0.13	15.10	-98.71	7.56	-24.52
89	5137.57	459.29	4909.07	739.52	7458.42	0.42	3.24	1.42	39.47	0.59	0.10	11.90	-97.60	6.52	-26.92
90	6128.45	187.83	2896.20	1347.78	3516.05	0.19	1.28	0.55	20.30	0.13	0.07	12.61	-99.56	5.30	-25.20
91	2388.73	131.08	2185.39	1194.10	702.99	0.26	1.55	0.76	2.41	0.04	0.00	11.06	-101.05	5.62	-21.56
92	5170.61	102.30	1669.03	797.24	1172.82	0.17	3.13	0.45	12.10	0.09	0.10	13.96	-100.51	6.80	-23.91
93	5187.88	136.41	2449.05	1595.61	1243.11	0.44	2.37	m	8.66	0.17	0.05	11.83	-99.74	6.31	-26.07
94	5137.76	85.32	1974.51	1471.66	476.98	0.20	2.14	0.57	6.68	0.11	0.04	13.43	-96.98	6.94	-25.34
95	3956.64	94.00	1695.05	1191.37	832.30	0.15	1.27	1.68	6.00	0.12	0.03	13.53	-94.94	6.88	-25.66
96	6708.02	183.60	3079.01	814.50	3982.74	0.21	1.21	0.41	26.41	0.12	0.10	14.28	-90.20	6.52	-24.82
97	6307.56	181.14	3039.62	756.87	3936.59	0.21	1.45	0.34	28.31	0.10	0.12	12.75	-103.16	5.78	-23.30
98	6133.71	173.41	5235.69	989.33	6329.29	0.27	1.29	0.42	41.55	0.19	0.27	12.03	-96.28	6.51	-23.81
99	6886.44	287.06	4727.83	1115.85	6539.11	0.57	2.77	0.46	34.00	0.15	0.11	13.81	-109.76	5.98	-21.30
100	9270.68	260.33	4626.55	702.90	6332.70	0.20	1.23	0.39	35.78	0.14	0.09	12.00	-105.77	7.17	-26.32
101	4609.03	185.02	3446.81	707.20	4577.71	0.19	1.80	0.33	26.58	0.10	0.09	12.92	-109.66	7.97	-25.24
102	4831.06	208.01	3813.25	742.85	5088.10	0.20	2.89	0.39	32.69	0.11	0.12	13.12	-106.08	7.86	-25.92
103	6557.37	227.19	4182.35	720.07	5475.00	0.22	3.09	5.74	43.51	0.15	0.24	11.77	-104.75	7.43	-25.97
104	8233.19	169.76	3252.59	913.50	3769.37	0.18	1.76	0.37	33.52	0.12	0.09	11.50	-106.03	7.82	-24.72
105	4368.94	240.48	4019.14	1416.44	5157.61	0.20	3.73	0.39	32.92	0.11	0.05	16.59	-81.76	6.66	-25.23
106	5952.06	227.93	3926.05	733.48	5426.54	0.18	1.89	0.35	28.56	0.13	0.10	12.87	-107.57	5.92	-24.71
107	4463.54	174.93	3244.82	772.24	4045.40	0.25	1.82	0.32	27.61	0.13	0.08	13.93	-107.71	7.00	-25.59
108	7570.72	253.18	4476.45	728.28	6292.82	0.22	1.94	0.43	32.51	0.17	0.13	13.08	-110.44	5.75	-24.94
109	5218.95	157.52	3015.19	709.99	3689.24	0.17	1.50	0.30	24.75	0.11	0.10	12.25	-110.65	4.34	-25.47

Sample	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
110	7011.99	295.80	4798.25	783.35	6983.67	0.18	1.17	0.43	34.64	0.14	0.11	13.35	-103.71	6.17	-24.52
111	6773.11	260.57	4534.60	728.43	6604.09	0.17	2.15	0.35	30.61	0.35	0.11	13.27	-114.46	6.20	-20.51
112	7841.77	388.80	4942.38	636.09	6997.49	0.35	3.73	0.72	30.41	0.27	0.04	12.41	-98.81	5.04	-25.00
113	5853.30	403.88	5442.16	806.00	7849.03	0.42	2.43	1.16	34.02	0.16	0.02	12.46	-105.52	6.48	-25.57
114	9140.76	239.42	4293.92	938.43	5679.37	0.20	2.07	0.29	32.65	0.10	0.10	14.41	-108.78	6.45	-23.28
115	6388.31	252.84	4117.06	656.10	5939.44	0.39	1.67	0.39	30.05	0.14	0.09	12.27	-105.30	6.87	-27.79
116	6843.35	247.58	4269.34	702.84	6063.44	0.24	1.80	0.40	31.07	0.16	0.11	13.19	-106.21	6.60	-24.87
117	6220.49	228.17	3930.48	706.56	5652.78	0.28	1.83	0.40	28.69	0.15	0.10	12.36	-106.58	6.07	-24.98
118	2615.61	172.51	3235.61	714.45	4141.77	0.15	1.32	0.30	25.39	0.10	0.08	14.42	-110.59	5.47	-26.31
119	5606.30	258.13	4627.44	675.76	6622.11	0.16	1.47	0.44	31.39	0.12	0.09	13.28	-107.77	6.95	-27.20
120	5077.98	193.55	3631.76	757.79	4713.27	0.53	1.52	0.34	29.51	0.08	0.08	13.40	-110.86	8.01	-26.28
121	6443.71	130.56	2528.96	657.03	3104.93	0.13	1.27	0.18	15.52	0.06	0.10	9.78	-120.13	7.28	-26.71
122	3134.29	314.25	5766.91	1085.86	8204.19	0.19	1.83	7.84	37.63	0.09	0.04	9.09	-108.08	7.74	-27.36
123	2457.69	360.85	6146.49	875.97	9079.90	0.22	1.77	8.89	47.45	0.11	0.22	10.37	-119.13	6.98	-25.81
124	9984.98	194.90	2920.56	596.06	3136.35	0.21	1.25	0.48	13.27	0.08	0.02	12.49	-95.60	5.47	-26.06
125	2492.51	187.02	3853.21	1140.13	4519.85	0.14	1.00	0.26	33.33	0.12	0.12	12.38	-96.87	5.91	-21.04
126	4294.71	343.29	5581.65	905.82	7975.25	0.21	4.13	6.68	51.95	0.11	0.20	9.94	-120.30	5.93	-18.59
127	4280.06	299.67	4501.13	813.72	6371.06	0.18	1.85	0.37	43.21	0.11	0.06	11.01	-112.88	5.20	-25.69
128	4439.89	185.93	2713.10	1075.66	2727.67	0.30	1.24	0.97	27.05	0.13	0.06	10.45	-105.92	6.20	-25.85
129	2482.66	145.50	2449.01	136.31	3564.20	0.24	9.50	0.31	22.48	0.08	0.07	10.83	-106.01	6.72	-25.33
130	6408.14	373.19	6034.04	1071.77	8640.70	0.19	1.72	4.39	49.28	0.22	0.27	10.20	-109.65	6.20	-27.83
131	3238.03	256.19	4638.39	900.68	7622.96	0.27	3.94	0.33	40.37	0.16	0.26	7.95	-122.53	4.27	-22.46

Appendix 4

Univariate statistical parameters for collected data

	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
# Missing	1	0	0	0	0	0	5	25	0	12	5	0	0	0	0
Mean	6416.83	263.23	4348.95	949.33	5851.80	0.32	2.23	1.68	31.20	0.19	0.10	13.22	-102.35	6.50	-25.61
Max	11337.69	536.56	6549.73	1764.87	10345.03	1.37	9.50	14.10	72.78	3.15	0.30	18.32	-68.86	8.01	-18.59
Min	2388.73	1.00	1.00	136.31	228.23	0.10	0.60	0.15	1.80	0.02	0.00	7.95	-122.53	4.27	-28.60
Range	8948.96	535.56	6548.73	1628.56	10116.80	1.27	8.91	13.95	70.98	3.13	0.29	10.37	53.67	3.74	10.01
Std. Dev	1991.36	95.61	1283.87	249.68	2270.08	0.17	1.30	3.02	10.71	0.31	0.06	1.51	6.93	0.76	1.82
Variance	3965506.00	9141.03	1648321.00	62341.14	5153279.00	0.03	1.69	9.10	114.79	0.10	0.00	2.28	48.02	0.58	3.31
RMS	6716.45	279.93	4533.12	981.37	6273.56	0.36	2.58	3.44	32.97	0.36	0.11	13.30	102.58	6.54	25.67
Skewness	0.28	0.09	-0.61	0.80	-0.75	2.45	2.53	2.41	-0.26	7.75	1.26	-0.10	0.61	-0.45	1.45
Kurtosis	-0.29	0.01	0.04	1.61	-0.05	10.16	9.68	4.89	1.79	69.74	1.82	1.78	4.40	0.28	2.60
Median	6300.24	260.33	4593.37	913.50	6453.34	0.27	1.88	0.43	32.51	0.13	0.09	13.29	-102.39	6.52	-25.81

Appendix 5

Correlation between individual variables[1]

	Na ppm	Mg ppm	P ppm	K ppm	Ca ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Se ppm	Mo ppm	δ18O ‰	δ2H ‰	δ14N ‰	δ13C ‰
Na	1	0.214	0.263	-0.145	0.276	0.047	-0.055	-0.232	0.182	0.189	-0.074	0.254	0.066	-0.061	-0.185
Mg	0.214	1	0.862	-0.071	0.827	0.299	0.348	0.210	0.584	0.247	0.088	0.090	-0.024	0.043	-0.222
P	0.263	0.862	1	-0.120	0.901	0.239	0.272	0.320	0.740	0.135	0.340	0.113	-0.131	0.082	-0.288
K	-0.145	-0.071	-0.120	1	-0.218	0.143	-0.120	0.188	-0.201	-0.083	-0.106	0.063	0.254	0.000	0.061
Ca	0.276	0.827	0.901	-0.218	1	0.230	0.322	0.252	0.806	0.123	0.368	0.104	-0.189	0.052	-0.284
Mn	0.047	0.299	0.239	0.143	0.230	1	0.237	0.185	0.120	0.042	-0.065	0.081	0.161	0.108	-0.126
Fe	-0.055	0.348	0.272	-0.120	0.322	0.237	1	0.116	0.379	0.023	0.146	0.102	0.075	0.007	-0.072
Cu	-0.232	0.210	0.320	0.188	0.252	0.185	0.116	1	0.337	-0.132	0.437	-0.096	-0.088	0.055	0.026
Zn	0.182	0.584	0.740	-0.201	0.806	0.120	0.379	0.337	1	0.015	0.541	-0.040	-0.263	0.013	-0.157
Se	0.189	0.247	0.135	-0.083	0.123	0.042	0.023	-0.132	0.015	1	-0.112	0.169	0.143	-0.036	-0.092
Mo	-0.074	0.088	0.340	-0.106	0.368	-0.065	0.146	0.437	0.541	-0.112	1	-0.070	-0.313	0.003	-0.028
δ18O	0.254	0.090	0.113	0.063	0.104	0.081	0.102	-0.096	-0.040	0.169	-0.070	1	0.576	0.128	-0.307
δ2H	0.066	-0.024	-0.131	0.254	-0.189	0.161	0.075	-0.088	-0.263	0.143	-0.313	0.576	1	0.122	-0.168
δ14N	-0.061	0.043	0.082	0.000	0.052	0.108	0.007	0.055	0.013	-0.036	0.003	0.128	0.122	1	-0.233
δ13C	-0.185	-0.222	-0.288	0.061	-0.284	-0.126	-0.072	0.026	-0.157	-0.092	-0.028	-0.307	-0.168	-0.233	1

[1] values in red have correlation coefficients ≥0.5

Appendix 6

Statistical Data for Isotopes

	$\delta^{18}O$ ‰			$\delta^{2}H$ ‰			$\delta^{14}N$ ‰			$\delta^{13}C$ ‰		
	IoI	**GB**	**Europe**	**IoI**	**GB**	**Europe**	**IoI**	**GB**	**Europe**	**IoI**	**GB**	**Europe**
Mean	13.55	13	10.4	-100.6	-106.3	-111.1	6.521	6.54	6.17	-25.82	-25.33	-24.69
Std. dev	1.266	1.212	1.382	5.599	6.203	9.687	0.6838	0.94	1.045	1.676	1.415	3.002
0ne Way ANOVA												
P value	< 0.0001			< 0.0001			0.3707			0.0819		
Are means significantly different? (P < 0.05)	Yes			Yes			No			No		
F-test	28.45			19.64			1			2.547		

Appendix 7

Summary of discriminant partial least squares model performances

Class Issue	Analyte	# loadings	Calibration		Prediction	
			Correct classification		Correct classification	
			IoI	Other class	IoI	Other class
Ire vs GB	Elements	1	6/9	5/9	2/74	14/14
Ire vs Europe		6	7/9	3/5	67/74	2/4
Ire+GB vs Europe		1	7/10	0/5	108/111	4/5
Ire vs GB+Europe		1	11/18	7/18	57/68	12/18
Ire vs GB	Isotopes	1	8/9	5/8*	71/74	12/14
Ire vs Europe		1	9/9	4/4*	81/87	2/4
Ire vs GB+Europe		1	14/18	8/13*	71/78	10/18
Ire+GB vs Europe		1	10/10	4/5	107/111	4/5
Ire vs GB	Elements + isotopes	1	8/9	5/8*	72/87	12/14
Ire vs Europe		1	8/9	3/4*	86/87	2/4
Ire vs GB+Europe		1	15/18	9/13*	71/78	10/18
Ire+GB vs Europe		1	8/10	1/5	110/111	3/5

*1 sample deleted

Appendix 8

Summary of SIMCA class-modelling results (p=0.05)

Analyte	Class Issue	# components	IoI	Other class	Sensitivity	Specificity
Elements	IoI vs GB	1	43/48	0/23	0.90	0.00
	IoI vs Europe	1	ditto	1/10	ditto	0.10
	IoI vs GB+ Europe	1	ditto	1/33	ditto	0.03
	IoI+GB vs Europe	1	82/111	4/5	0.74	0.80
Isotopes	IoI vs GB	4	38/48	8/23	0.79	0.35
	IoI vs Europe		ditto	7/10	ditto	0.70
	IoI vs GB+ Europe		ditto	15/33	ditto	0.45
	IoI+GB vs Europe	1	107/111	3/5	0.96	0.60
Elements + Isotopes	IoI vs GB	4	46/48	2/23	0.96	0.09
	IoI vs Europe		ditto	5/10	ditto	0.50
	IoI vs GB+Europe		ditto	7/33	ditto	0.21
	IoI+GB vs Europe	1	91/111	1/5	0.82	0.20

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