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TITLE: Multivariate analysis of Scotch whisky by total reflection x-ray fluorenscence and chemometric methods: a potential tool in the identification of counterfeits.

YEAR: 2017

Publisher citation: SHAND, C.A., WENDLER, R., DAWSON, L., YATES, K. and STEPHENSON, H. 2017. Multivariate analysis of Scotch whisky by total reflection x-ray fluorenscence and chemometric methods: a potential tool in the identification of counterfeits. Analytica chimica acta [online], 976, pages 14-24. Available from: https://doi.org/10.1016/j.aca.2017.04.041

OpenAIR citation: SHAND, C.A., WENDLER, R., DAWSON, L., YATES, K. and STEPHENSON, H. 2017. Multivariate analysis of Scotch whisky by total reflection x-ray fluorenscence and chemometric methods: a potential tool in the identification of counterfeits. Analytica chimica acta, 976, pages 14-24. Held on OpenAIR [online]. Available from: https://openair.rgu.ac.uk

Accepted Manuscript

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PII: S0003-2670(17)30531-7

DOI: 10.1016/j.aca.2017.04.041

Reference: ACA 235196

To appear in: *Analytica Chimica Acta*

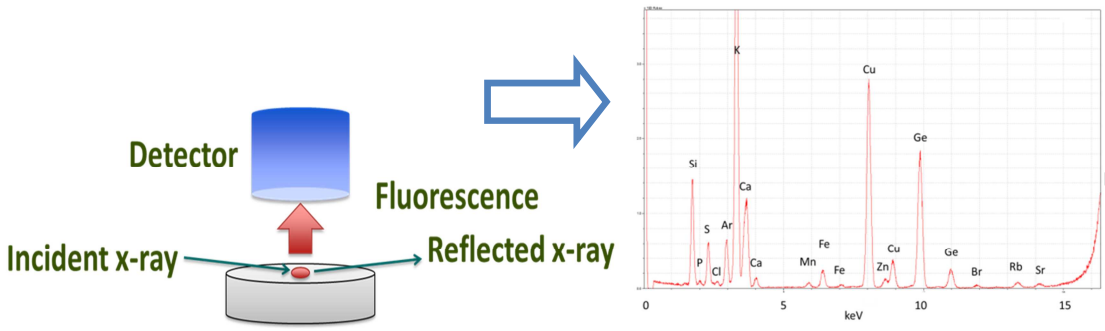
Received Date: 18 January 2017

Revised Date: 13 April 2017

Accepted Date: 20 April 2017

Please cite this article as: C.A. Shand, R. Wendler, L. Dawson, K. Yates, H. Stephenson, Multivariate analysis of Scotch whisky by total reflection x-ray fluorescence and chemometric methods: A potential tool in the identification of counterfeits, *Analytica Chimica Acta* (2017), doi: 10.1016/j.aca.2017.04.041.

Graphical abstract



Multivariate analysis of Scotch whisky by total reflection x-ray fluorescence and chemometric methods: a potential tool in the identification of counterfeits

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ABSTRACT: Most methods used in the identification of counterfeit whisky have focused on the profiling of volatile organic congeners determined by gas chromatography. We tested the use of total reflection x-ray fluorescence (TXRF) for trace element analysis of whisky and application of the data as a potential tool in the identification of counterfeit samples. Twenty five whiskies that were produced in different regions of Scotland or were blends, 5 counterfeit whiskies, 1 unmatured grain whisky, and 1 matured grain whisky were analysed for 11 elements (P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br and Rb). The effect of cold plasma ashing with oxygen on whisky residues evaporated on the TXRF reflector on the instrument performance was investigated. Cold plasma ashing with oxygen reduced beam scatter and improved the limits of detection but was ultimately deemed unnecessary. The element concentration data for whisky obtained by TXRF (after log transformation) was compared with the values obtained by inductively coupled plasma spectroscopy and

showed correlation values (R^2) ≥ 0.942 for K, Mn and Cu: ≥ 0.800 for Ca, Fe and Rb; and ≥ 0.535 for P, S and Zn. The range of concentration values for individual elements was variable and principal components analysis of the elemental concentrations partially differentiated the whiskies by region but showed clear separation of the counterfeit samples from the other samples. Using the principal component scores of the elemental concentration data, linear discriminant analysis also distinguished the counterfeits from the other samples.

Keywords: Counterfeit; plasma ashing; trace elements; whisky; x-ray fluorescence

1. Introduction

Scotch whisky makes up around one quarter of the UK's total food and drinks exports and in 2015 was valued at £3.95 billion for the UK balance of trade. Scotch whisky is exported to around 200 markets worldwide and supports over 40,000 jobs across the UK [1]. Due to its large market and relatively high prices, Scotch whisky counterfeiting is common, especially with blends. Not only does counterfeiting defraud consumers and producers, it poses underlying health risks. The methods used to identify whisky type, composition and authenticity have been reviewed [2]. Most methods have focused on the profiling of volatile organic congeners (VOCs) determined by gas chromatography [3, 4]. Other analytical methods include the determination of the stable isotope ratios of carbon, hydrogen or oxygen [5-7]. These chromatographic and isotopic methods generally require expensive equipment and highly trained operators. Testing of the authenticity of Scotch whisky in field situations requires methods that

use portable equipment. To this end, MacKenzie and Aylott [8] developed a small battery powered uv / visible spectrophotometer that could distinguish (some) counterfeit whiskies. McIntryre *et al.* [9] used attenuated total reflectance (ATR) in the mid infrared spectral region with a probe directly in the sample to assess ethanol concentration and diamond ATR with dried residues to distinguish authentic and counterfeit whiskies. Ashok *et al.* [10] used a small optofluidic device with an IR source to rapidly determine the alcohol concentration in Scotch whiskies and to classify them based on age, type and cask. Mignani *et al.* [11] used a combination of optical absorption and fluorescence spectroscopy and multivariate analysis to differentiate distinctive single-malt Scotch whiskies from commercial-grade blends, and for classifying them according to the region of production.

In addition to VOCs and other organic materials that can be used as markers to aid identification of origin, alcoholic drinks also contain trace elements derived from the raw materials, production process equipment, storage vessels and additives [12] but compared with the determination of organic markers little application of trace elements to the identification of counterfeit whisky has been tested. Anodic stripping voltammetric determination of metals in whisky, without prior treatment, has been used to measure the concentration of Cu, Zn and Pb in unspecified whiskies [13]. Graphite furnace atomic absorption has been used to measure the concentration of Cu, Zn, Pb, Ni, Fe, Ca. Mg and Na in 35 scotch whiskies and from the data it was concluded that the 'fingerprint' of the metals could not be used to identify different regions of whisky production but Cu concentration alone could be used to distinguish a malt whisky from a blended or grain whisky [14]. Using inductively coupled plasma

(ICP) spectroscopy for analysis and canonical discriminant analysis and classification binary tree statistical methods, Kokkinofta *et al.* [15] found Mg, Zn and Cu concentrations distinctive parameters that could be used to differentiate zivania, a traditional alcoholic drink from Cyprus, from other spirits, believed to be related to the unique geology and climatic conditions. The health risks related to the trace metals in a range of distilled alcoholic beverages, including several whiskies from Scotland, have been suggested [16].

Total reflection x-ray fluorescence (TXRF) with small, portable instruments containing miniature x-ray tubes and Si drift detectors is a relatively new development of XRF spectrometry that has been used for elemental analysis of beverages such as tea, coffee and wine [17] but its application has not been reported within the Scotch whisky analysis sector. Compared with conventional XRF, TXRF has detection limits which are 3-4 orders of magnitude improved. Total reflection x-ray fluorescence spectroscopy is capable of simultaneously quantifying the concentration of many metals and non-metals with the exception of “light elements” and those forming part of the x-ray tube. In comparison to the use of ICP, TXRF spectroscopy has less spectral interference, has micro-analytical capability (typical sample volume of 10 µL), can use a single element for the standardisation of all elements, and is cheaper to buy and operate. It can also simultaneously analyse anionic (*e.g.* halogens) and cationic elements (*e.g.* metals). Total refection x-ray fluorescence spectroscopy has been applied to the trace metal analysis of some alcoholic and non-alcoholic beverages [18]. For example, TXRF spectroscopy has been used for the analysis of Madeira wine following pretreatment to remove residual organic matter [19] or table wine directly

[20-24]. Total reflection XRF spectroscopy has been applied to the analysis of distilled drinks. For spirits containing sufficient quantities of sugars that result in a viscous residue, preliminary evaporation, ashing at 500°C, and leaching the residue with nitric acid was required before the sample could be applied to the TXRF support and analysed for Fe, Cu and Zn [25]. In contrast, Capote *et al.* [26] were able to directly analyse commercial spirits and homemade "firewater" for the same elements.

When evaporated, whisky leaves a small deposit of non-volatile organic matter, which can cause undesirable scatter of the x-ray beam into the TXRF detector. The detection limits in TXRF spectroscopy depends *inter alia* on the signal-to-noise ratio at the detector. A range of ashing methods have been applied in TXRF spectroscopy to remove the organic sample matrix [27]. Cold plasma ashing (CPA) with oxygen is attractive because in comparison with wet ashing, CPA can be expected to minimise the introduction of extraneous contaminating material, but there is a potential danger of loss of volatile elements. Cold plasma ashing has been used to remove organic matter from a variety of organic substrates in TXRF spectroscopy-related studies including zebrafish [28], nematodes [29] and microcrustaceans [30].

Chemometric methods for determining the authenticity of wine have been extensively studied [31]. For the identification of grape variety, analyses of volatile compounds are often employed, whereas for the classification of geographical region of production, minerals are often employed [32]. Multivariate methods including principal component analysis, cluster analysis, discriminant analysis, multiple linear regression, and ANOVA have all been successfully applied [33, 34]. To our knowledge, TXRF spectroscopy coupled with

multivariate statistical methods have not been applied to the analysis of Scotch whisky. Our study reports on the elemental analysis of a small sample-size of malt, grain and blended Scotch whiskies by TXRF for Cu, Zn, Fe, Ca, S, Cl, K, Mn, P, Rb and Br and the use of the data for the determination of authenticity and provenance. These elements were selected because they were associated with the whisky production process and their concentrations in most samples were above the limits of detection by TXRF. We also assessed the use of CPA to remove residual organic matter from dried whisky residues on the quartz TXRF spectroscopy support, with the aim of reducing x-ray beam scatter and / or inter-element interferences.

2. Materials and methods

2.1. Whisky

The whisky producing regions of Scotland can be divided in a number of ways [35]. We divided the samples into the four general regions (Highland, Speyside, Islay and Lowland), see (Fig. S1). Twenty five whiskies from reputable market places that were produced in the different regions of Scotland, 2 grain whiskies and 5 whiskies known to be counterfeit were analysed (Table 1). Of the 25 from the market place, 8 were blends of unknown local origin. The counterfeits and grain whiskies were from sources which remain anonymous. The samples were analysed without filtration or dilution.

2.2. TXRF spectrometry

A benchtop TXRF spectrometer (Bruker S2 PICOFOX^{TM}, Germany) with an air-cooled X-ray tube and a Mo target was used (tube voltage 50 kV, current 600 µA). The instrument has a 25-place auto sampler and we used quartz carrier discs throughout. Each sample was prepared in triplicate (*i.e*., on three separate discs) and the live time for analysis was 1000 s. The quartz carriers were cleaned and siliconised according to the manufacturer's recommendations, as described by Towett *et al.* [36] and checked for any contamination by analysing the discs for 1000 s, a sufficient period to ensure levels of contamination by elements such as Fe and Zn are absent at appropriate concentrations expected in whisky. Siliconised discs showing net counts for $K\alpha_{1,2}$ lines of P > 25, S > 5, Cl > 110, K > 20, Ca > 80, Mn > 80, Fe > 260, Cu> 100, Zn> 130, Br > 10 or Rb > 1 were returned for another cleaning and check cycle. A standard solution of 0.5 mg Ge L^{-1} was freshly prepared from a stock 1000 ± 3 mg Ge L^{-1} in 2 % nitric acid purchased from CPI International, Amsterdam. As far as possible, preparations were carried out in a laminar air flow cabinet to minimise airborne contamination.

Using one selected whisky (sample 30, Glenrothes, Table 1), two method of applying the sample to the quartz carrier discs were investigated. Sample 30 was chosen as it had an average composition with detectable concentrations for all the selected elements. In the first method, 10 µL of the whisky was applied to the disc, immediately followed by 10 µL of the 0.5 mg Ge L^{-1} standard directly on top of the whisky droplet, followed by evaporation on a hot plate at 60 ˚C. In the second method, equal volumes of the whisky and Ge standard solution were mixed before applying two 10-µL drops directly together on the disc, followed by evaporation on a hot plate at 60 ˚C. The TXRF spectra were analysed using SpectraMax software and in addition to the signals from Si (from the support and

siliconising fluid), Ar (from the air) and Mo (from the tube) were generally assigned to P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br and Rb, although occasionally other trace elements (*e.g.* Ni) were included but are not reported here. Deconvolution used the method "profile bayes" with a normal fit. The quality of the fit was assessed visually and using the value of the standardised square sum of the differences between the measured and the calculated deconvolution intensities. Preliminary analysis of the whiskies, without the addition of Ge, showed that the samples did not display any corresponding Ge signals indicating the suitability of Ge as an internal standard. The amount of Ge standard added to the carrier as internal standard was selected to provide a peak area approximately corresponding to that of the signal from Cu. In TXRF studies, Ga is often the preferred standard but we chose Ge as the signals are further removed from those due to Zn, which we considered an important element in discrimination in our study.

2.3. Cold plasma ashing (CPA)

An Emitech K1050 X Plasma Asher with high purity oxygen (> 99.95 %) at a pressure of 70 Pa was used to oxidise the whisky residues (left from the evaporation of the 10 µL sample) on the quartz TXRF discs. To minimise physically disturbing the sample and potential airborne contamination, a Millipore Millex-HN Nylon 0.45 µm cassette filter was fitted to the air inlet tube leading to the chamber. The whisky residues on quartz discs were ashed at a power of 75 W for 2 h. The capability of the asher to destroy organic matter was tested on 40 mg sucrose powder held in an aluminium cup at a lesser power of 25 W. The residual weight was recorded at intervals up to 2 h. The experiment was performed in duplicate.

193 To further investigate the effect of CPA on whisky residue we used Fourier transform
194 infrared (FTIR) with a Bruker Vertex 70 spectrometer equipped with a diamond attenuated
195 total reflectance (DATR) accessory and one selected peaty whisky (sample 21, Talisker,
196 Table 1). To provide sufficient sample mass, 10 mL of the whisky was evaporated at room
197 temperature (approximately 25 °C) in a glass vessel. A portion of the residue obtained was
198 transferred onto the DATR device with a spatula and the sample scanned between
199 wavenumbers 400-4000 cm^{-1}. The remainder was subject to CPA and the FTIR analysis
200 repeated.
201
202 *2.4. ICP Analysis*
203
204 Eighteen of the 32 samples were analysed by ICP. The range included blends, counterfeits,
205 grain, island, and lowland samples as listed in Table 1. The other samples were not
206 analysed because of the restricted volumes available. Twenty-milliliter aliquots of whisky
207 were evaporated to near dryness in 50-mL beakers on a hot plate set at 100 °C. The
208 residual solution was left to evaporate at room temperature (approximately 25 °C) and the
209 solid residue remaining treated with 2.56 mL of trace-analysis grade 15.6 M nitric acid and
210 warmed to aid dissolution of solids. The mixture was diluted with high purity, deionised
211 water, warmed to ensure that all the solids were dissolved and made up to 20 mL with
212 water in a volumetric flask. The solutions were analysed by ICP-mass spectroscopy (Agilent
213 7700) for Mn and Rb, and ICP-optical emission spectrometry (Optima) for Mg, P, S, K, Ca,
214 Fe, Cu and Zn using matrix matched standards. A composite sample comprising of equal
215 volumes of ten whiskies (Sample no. 2 - 7, 18, 20, 22 and 23 listed in Table 1) was
216 evaporated and the residue dissolved in nitric acid as described previously. The extract of

the composite sample was used to determine the statistical errors associated with the ICP analysis.

2.5. Statistics

In total we analysed 32 samples of whisky. The set comprised of 25 whiskies purchased from reputable sources, 2 grain, and 5 counterfeit samples. Because the availability of counterfeits was beyond our control and we could only obtain a maximum of 5 counterfeit samples, the experimental design was not ideally balanced with respect to the total number of samples.

Statistical analyses were carried out using Minitab™ versions 16 and 17, and Microsoft Excel 2010. Error terms (±) where presented refer to one standard deviation. The normality of data was tested using the Kolmogorov Smirnov test. The limit of detection (LOD) for elements in whisky measured by TXRF were calculated according to Equation 1, where C_i is the concentration of the element, N_i is the area of the fluorescence peak in counts, and N_{BG} is the background area subjacent to the fluorescence peak [37].

$$\boldsymbol{LOD_i = (3 \times C_i \times \sqrt{N_{BG}})/N_i} \qquad \text{Equation 1}$$

The LODs for ICP spectroscopy were determined differently using single element standards in dilute nitric acid but used a similar 3-sigma approach. Principal component analysis used the correlation matrix, and dendograms showing linkages between variables used Euclidean distance: in both cases the trace element concentration data was first normalised by

logarithmic transformation. Linear discriminant analysis used the scores from the PCA and cross validation was applied.

3. Results and discussion

3.1. Application of whisky sample to the quartz support

A comparison of the element concentration data obtained by TXRF spectroscopy following the two different sample application methods (*i.e.*, mixing equal volumes of the whisky and standard Ge solution directly on the quartz disc, and that obtained by premixing equal volumes of the whisky (sample 30, Glenrothes, Table 1) with the Ge standard solution before application to the quartz disc is shown in Table 2. The measured concentration values for the elements were not statistically different between the sample treatment methods ($p = 0.05$), and were close to those determined previously (Table 1). The variation (standard deviation) between replicates was often better for the premixing procedure and this method was adopted as our standard procedure. An example TXRF spectrum, labelled with the elements measured, for the Glenrothes whisky is shown in Fig. 1.

3.2. Cold plasma ashing and TXRF background

Cold plasma ashing of powdered sucrose displayed an approximately exponential weight loss pattern and > 90 % of the initial mass was lost in 2 h. We assumed that 2 h reaction time at 75 W would be sufficient to remove much smaller but likely more recalcitrant,

residual organic matter from whisky residues dried on the disc and improve the signal to noise ratio and / or help reduce inter-element x-ray interference if such effects were to exist within the thin film.

To monitor the effect of CPA on the TXRF background signal we inspected the background values under the Cu fluorescence signals near 8.03 keV. At this energy position, clean quartz discs had average background counts before and after siliconisation of 211 ± 14 and 214 ± 13, respectively. After adding the internal Ge standard the background counts increased to 332 ± 22. The error terms relate to ± standard deviation for 15 separate discs. With one exception, the background under the Cu fluorescence signal for whisky and the internal standard on the siliconised discs was between 454 and 1716 counts (average 997). After cold plasma ashing the background count was reduced to values between 365 and 1460 (average 682). The exception was one of the counterfeit whiskies (sample 11, Table 1), which left an easily visible light-coloured, residual spot with a relatively high background of 12,829 ± 232 counts. The background for this sample was reduced to 981 ± 479 (n = 3) counts by CPA.

The FTIR absorbance spectra of sample 21, Talisker (Table 1) before and after CPA are compared in Fig. S2. Both spectra have peat-like character [38]. Prior to CPA the whisky residue was brown and had characteristic absorbance peaks for wax or fatty acids (2924, 2854 cm^{-1}), carboxylic acid (broad peak centred on 2700 cm^{-1}), carboxylic acid salts (1604 cm^{-1}), lignin (1516 cm^{-1}), and carbohydrates in the form of polysaccharides (1034 cm^{-1}). The organic residue from sample 21 was not totally decomposed by CPA but there was a reduction in absorption intensities particularly for wax (2927, 2850 cm^{-1}) and carbohydrates

in the form of polysaccharides (1041 cm^{-1}).

Cold plasma ashing had little effect on the measured concentration of P, S, K, Ca, Cu, Zn and Rb determined by TXRF but there were large differences for the measured concentrations of the halogens Cl and Br, and for Mn and Fe (Fig. 2). There was an apparent loss of Br but a gain of Cl. The offset and scatter of the data for Mn and Fe indicate contamination by these elements in the plasma ashing chamber. After logarithmic transformation, the element concentration data were normally distributed (Kolmogorov Smirnov test, $p = 0.05$) apart from minor deviation for P and Mn in the ashed samples. The correlation coefficient (R^2) for the transformed data was ≥ 0.963 for S, K, Ca, Cu, Zn and Rb, but was considerably poorer for the other elements (P, 0.894; Cl, 0.690; Mn, 0.181; Fe, 0.293; and Br, 0.589). A paired t-test showed that there were statistically significant differences ($p \leq 0.05$) between the mean values of the log transformed data for all elements apart from P. Because of the marginal gain in reducing the background intensity, and the potential losses or gains we considered CPA an undesirable step in future work.

3.3. TXRF and ICP limits of detection

The LOD for TXRF measurements varied between elements and between samples but generally improved as the atomic number increased. Although not strictly comparable because the TXRF LODs were determined with real whiskies, whereas ICP LODs were determined with single element standards, the performance of ICP was better than TXRF for P detection but similar for the other elements measured (Table 3). Using blank discs Lofthouse *et al.* [39] reported similar LODs (mg L^{-1}) by TXRF for Mn (0.003), Fe (0.002), Cu

(0.002) and Zn (0.001). For the TXRF analysis of a freshwater reference sample using Mo – Kα radiation Stosnach [40] reported the following detection limits (mg L^{-1}); K (0.0069), Ca (0.0049), Mn (0.0013), Fe (0.0008), Cu (0.0006) and Zn (0.0004).

3.4. TXRF analysis of whisky without CPA

The concentrations of the elements in the whiskies determined without CPA are shown in Table 1 along with their mean, median, maximum and minimum values. Phosphorus concentrations were below the limit of detection for 10 samples and Rb concentrations were below the limit of detection for 3 samples. The concentration ranges for individual elements were variable, *e.g.*, K concentrations varied over two orders of magnitude being between 0.336 and 37.7 mg L^{-1}. The median element concentrations can be grouped: [K > S > Ca (6.2 to 1.0 mg L^{-1})] >> [Cu > Cl > P > Fe (0.25 to 0.08 mg L^{-1})] >> [Zn > Mn > Rb > Br (0.03 to < 0.004 mg L^{-1})].

3.5. Comparison of TXRF and ICP analysis of whisky

The element concentrations in the eighteen whiskies measured by ICP spectroscopy were below the LOD for P in Samples 9 and 15; Mn in Sample 25; Fe in Samples 8, 10, 11, 12 and 25; Zn in Samples 11 and 12; and Rb in Samples 9, 11, 24 and 25. No data was available for Cl and Br by ICP spectroscopy because ICP analysis of the halogen elements requires a different, non-routine set-up. Various substitution approaches for dealing with values below the LOD exist [41] and to determine the correlation between ICP and TXRF, values below the LOD were substituted with half of the LOD. Using the 1:1 line as a guide, a

comparison of TXRF and ICP derived concentrations for P, S, K, Ca, Mn, Fe, Cu, Zn and Rb in the whiskies (Fig. 3) indicated good agreement between the TXRF and ICP data. After log transformation the data were normally distributed (Kolmogorov Smirnov test, $p = 0.05$) and the correlation coefficient (R^2) for the transformed data was ≥ 0.942 for K, Mn and Cu: ≥ 0.800 for Ca, Fe and Rb; and ≥ 0.535 for P, S and Zn. The systematic offset and data scatter for Fe, indicates the possibility of contamination, spectral interference or un-recognised x-ray interactions between elements. In general the standard deviations of the TXRF analysis (for 3 separate analyses) were relatively small and less than the size of the symbols used in the graph. The ICP concentration data were not individually replicated but triplicate analysis of the composite whisky sample showed that the relative percentage standard deviations (% RSD) associated with the ICP analysis were ≤ 2.77 % for P, S, K, Ca, Cu, Zn and Rb. The percentage RSD was greater for Fe (5.24 %) and Mn (3.15 %). With the exception of Fe, the error terms associated with the ICP analysis are smaller than the size of the symbols used in Fig. 3. The concentrations of Mn (0.020 mg L^{-1}) and Fe (0.093 mg L^{-1}) measured in the composite sample by ICP were small and may in part explain the relatively large errors associated with their measurement. Although precautions were taken to avoid contamination and the siliconised discs were checked before loading the samples, contamination remains a possibility. Stosnach *et al*. [40] reported overestimation of Fe in reference water samples analysed by TXRF, most probably caused by contamination. We checked blank discs left in the instrument and in the laminar air flow cabinet, and the composition of the internal standard, but could not pinpoint a source of contamination. Spectral interferences from escape or pile-up peaks were considered [42]. The TXRF software was programmed to automatically correct for escape peaks. With Si-based detectors, escape peaks affecting Fe (6.392 and 6.405 keV) could arise from Cu (8.027 and

8.046 keV) resulting in peaks at 6.287 and 6.307 keV, respectively, but there was no indication of additional shoulders or peaks at the predicted positions.

3.6. Statistical analysis

In the statistical analysis we make use of multivariate methods including principal component analysis (PCA), cluster observations visualized in the form of dendograms, and linear discriminant analysis. The PCA method is a commonly used multivariate technique and aims to reduce the dimensionality of the data set consisting of a large number of interrelated variables (in this case elemental concentration data for 11 elements) while retaining as much as possible of the variation present in the original set. The reduction in dimensionality is achieved by transforming to a new set of variables, the principal components, which are uncorrelated, and ordered so that the first few components retain most of the variation present in the original variables [43]. The PCA method has found wide application in grape and wine analysis [44]. For linear discriminant analysis (LDA), we used the scores for the first six principal components of the elemental data to reduce the number of variables and applied cross validation to classify the whiskies into type (blend, counterfeit, grain,) or regional (Highland, Island, Lowland, Speyside) categories. Although similar to PCA in so far that they both derive linear combinations of variables, LDA maximizes the component axes for class-separation and explicitly attempts to model the difference between them [45].

Principal component analysis used the normally-distributed, log-transformed data. The eigenvalues for the first three components were PC1, 5.24 (47.6 % of variance); PC2, 2.33

(21.2 % of variance); and PC3 1.19 (10.8 % of variance). The Mahalanobis distance plot showed there were no outliers. The first two components (Fig. 4) accounted for 68.8 % of the variance and indicated that the counterfeit samples could be distinguished from the others on the basis of the profile of their trace element composition. The second component was particular important in separating the counterfeit samples from the whiskies of Scottish origin, with all five counterfeit samples separating out (Fig. 4). The three dimensional plot of the scores for the first three principal components shows the effect of the third component on the separation of classes. The loading plot for the first two components (Fig. 5) showed the counterfeit whiskies to have higher overall concentrations of S, Ca or Br and lower overall concentrations of Cu, Mn, K or Rb, as shown in Table 4. The scores for the third principal component showed positive values for Zn (0.552), Fe (0.485), S (0.228), Cu (0.132) and Rb (0.083), and negative values for Cl (-0.407), Ca (-0.300), Br (-0.245), P (-0.216), K (-0.110) and Mn (-0.097). For the log transformed data there were statistically significant positive correlations ($p < 0.05$) between the concentrations of many elements, but the correlation was especially strong between Rb and K ($R = 0.933$), between Mn and K ($R = 0.899$) and correspondingly between Mn and Rb ($R = 0.892$). The dendogram related to the correlation coefficient distance of the elements is given Fig. 6. Although there is chemical similarity between the Group 1 elements Rb and K, there is no obvious chemical or geochemical connection between Mn and K or Rb. The dendogram (Fig. 7) shows the grouping of the five counterfeit samples numbered 9 to 11. In this cluster observation method, the counterfeit samples are not completely distinguished and have similarity to some of the blends (samples 4, 6, 7, 8) and to the unmatured grain whisky (sample 15) and is likely related to the inclusion of all the data, whereas analysis by principal components is selective. The variation in trace elements between individual bottles of the same brand of

409 whisky was not tested. Since PCA indicated some regional distinction between whiskies it
410 can be inferred that the variation between bottles of the same brand are relatively smaller
411 than that associated with the same brand.
412
413 Linear discriminant analysis using the scores from first three components of the PCA and
414 cross validation, correctly classified all five counterfeit samples, seven of the eight blends,
415 five of the nine Speyside, two of the four Island, but failed to correctly identify the grain,
416 Highland or Lowland whisky samples. The thirteen samples not correctly classified were 5,
417 14-18, 21-23, 27, 28, 30 and 32 (see Table 1). Increasing the number of PCs scores included
418 in the LDA analysis from three to six gave similar results with counterfeits correctly
419 classified, although the number of correct classifications fell from nineteen to sixteen of the
420 thirty two whisky samples.
421
422 The number of whisky groups (n = 7) compared with the total number of samples (n = 32)
423 was quite large (see descriptors in Table 1). The three dimensional PCA plot (Fig. 4) shows
424 that the positions of Highland, Island, Lowland and Speyside samples on the graph are not
425 well separated. After combining these categories into one larger group, LDA correctly
426 classified all five counterfeit samples, seven of the eight blends, fourteen of the seventeen
427 in the new combined group but failed to correctly classify the two grain samples. The six
428 samples not correctly classified were 5, 14, 15, 21, 27 and 31 (see Table 1). Increasing the
429 number of PCs scores included in the LDA analysis from three to six gave similar results but
430 in this case only four out of the five counterfeits were correctly classified. Counterfeit 5
431 (sample 13 in Table 1) was classified as grain and the overall number of correct
432 classifications fell from nineteen to sixteen of the thirty two whisky samples.

4. Conclusions

Although there are notable examples of the use of TXRF for the determination of trace elements in spirituous beverages [25, 26] there are no examples of the use of TXRF for the analysis and provenance of Scotch whisky. The concentration of a range of trace elements in microliter volumes of Scotch whisky can be determined directly by TXRF without pretreatment. The residual material left after evaporation is physically stable and evenly distributed on the quartz support. Although cold plasma ashing reduces the background signal by around one third, the extra step may result in contamination from material in the ashing chamber, and was thus deemed unnecessary. The results of the limited range of samples we analysed showed that the element concentrations vary considerably between whisky samples. The TXRF instrument is small and portable between laboratories, samples require little preparation apart from the addition of an internal standard, samples of around 10 µL volume can be analysed, and the method provides an alternative to the use of laboratory based inductively coupled plasma spectroscopy or other multi-element techniques. However, the analysis of so called “light” elements by the current TXRF methodology is restrictive and some elements may be lost by volatilisation during the evaporation stage of sample preparation. Statistical procedures using PCA and LDA were able to correctly classify counterfeit counterfeit whisky samples although some caution is expressed because of the unbalanced design of the experiment in relation to the relatively small number of counterfeit whisky samples analysed (n = 5) compared with the total (n = 32).

Acknowledgements

We thank the Scottish Government Rural and Environmental Science and Analytical Services (RESAS) Division for partly funding the work and the anonymous donors of the counterfeit whisky samples. We thank Tony Fraser and Jean Robertson for recording the FTIR spectra and providing help with interpretation of the spectra and Betty Duff for statistical advice.

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586 Table 1

587 Whisky sample description, and trace element concentrations determined by TXRF. Units

588 are mg L^{-1} and "<" values refer to the limit of detection.

589

Sample no.	Descriptor	Distillery	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Br	Rb
		Ballie Nicol											
1	Blend	Jarvis	0.152	1.10	0.173	7.86	1.45	0.032	0.027	0.186	0.015	0.002	0.006
2[a]	Blend	Bells	0.653	1.58	0.238	4.93	1.40	0.019	0.110	0.242	0.021	0.005	0.003
3[a]	Blend	Chivas	0.375	0.809	0.193	4.31	1.22	0.019	0.044	0.196	0.007	0.003	0.002
4[a]	Blend	Dewars	0.121	1.16	0.157	3.20	1.14	0.011	0.050	0.189	0.018	0.003	0.003
5[a]	Blend	Johnnie Walker	<0.326	1.09	0.180	5.48	0.526	0.018	0.103	0.286	0.020	0.002	0.002
		The Famous											
6[a]	Blend	Grouse	<0.145	0.615	0.097	2.74	0.416	0.009	0.050	0.208	0.007	0.002	0.001
		White and											
7[a]	Blend	Mackay	0.067	0.576	0.151	2.36	0.745	0.012	0.047	0.159	0.019	0.003	0.002
8[a]	Blend	William Grant	0.239	0.748	0.147	2.84	0.976	0.010	0.021	0.137	0.020	0.003	0.002
9[a]	Counterfeit	Unknown 1	<0.089	4.06	0.066	0.336	1.24	0.007	0.154	0.085	0.038	0.005	<0.001
10[a]	Counterfeit	Unknown 2	<0.088	14.7	0.072	1.23	1.40	0.006	0.025	0.052	0.018	0.004	0.001
11[a]	Counterfeit	Unknown 3	<0.279	15.9	<0.083	0.811	1.36	0.006	0.057	0.038	0.016	<0.002	<0.002
12[a]	Counterfeit	Unknown 4	0.320	22.1	0.596	2.32	1.78	0.008	0.019	0.038	0.015	0.068	<0.001
13[a]	Counterfeit	Unknown 5	<0.120	26.1	0.071	2.37	1.63	0.010	0.082	0.187	0.194	0.012	0.005
14[a]	Grain	Grain matured	0.034	2.23	0.252	6.44	1.04	0.013	0.115	0.174	0.019	0.004	0.006
		Grain											
15[a]	Grain	unmatured	<0.084	5.53	0.113	3.25	1.35	0.012	0.076	0.164	0.046	0.010	0.003
16	Highland	Glengoyne	1.04	5.57	0.343	24.2	0.857	0.023	0.197	1.251	0.041	0.004	0.016
17	Highland	Glenmorangie	<0.126	0.796	0.245	6.95	0.859	0.035	0.025	0.523	0.011	0.003	0.006
18[a]	Island	Bowmore	0.914	6.67	0.316	21.1	0.868	0.037	0.148	0.548	0.032	0.007	0.018
19	Island	Bruichladich	1.63	5.48	0.697	36.5	4.13	0.038	0.288	0.587	0.066	0.034	0.039
20[a]	Island	Bunnahabbain	2.24	7.54	1.35	36.2	2.12	0.051	0.184	0.580	0.057	0.014	0.037
21	Island	Talisker	0.034	4.85	0.362	5.67	0.607	0.018	0.070	0.277	0.033	0.003	0.006
22[a]	Lowland	Auchentoshen	0.169	1.46	0.417	11.7	0.681	0.042	0.128	1.32	0.037	0.006	0.012
23[a]	Lowland	Glenkinchie	<0.108	2.45	0.176	7.76	0.738	0.031	0.106	0.434	0.022	0.002	0.007
24	Speyside	Balvenie	0.695	3.85	0.120	20.3	0.765	0.031	0.121	0.380	0.035	0.005	0.024
25	Speyside	Craigellachie	0.096	0.819	0.177	6.11	0.633	0.024	0.094	0.239	0.025	0.005	0.006
26	Speyside	Dufftown	0.883	4.64	0.130	14.0	1.05	0.030	0.078	0.533	0.024	0.002	0.014
27	Speyside	Glen Elgin	<0.115	1.35	0.404	9.27	1.40	0.031	0.046	0.195	0.029	0.006	0.009
28	Speyside	Glenburgie	2.00	7.91	0.185	37.7	1.65	0.053	0.134	0.198	0.043	0.008	0.026
29	Speyside	Glennfiddich	0.317	2.72	0.344	12.4	0.660	0.029	0.132	0.519	0.193	0.004	0.013
30	Speyside	Glenrothes	0.953	4.11	0.399	16.7	1.83	0.041	0.137	1.030	0.029	0.007	0.014
31	Speyside	Knockando	0.051	1.03	0.191	5.14	0.605	0.017	0.094	0.432	0.020	0.008	0.005
32	Speyside	Linkwood	0.276	1.05	0.207	6.22	1.01	0.020	0.064	0.769	0.019	0.004	0.006
		Mean	0.437	5.01	0.269	10.3	1.19	0.023	0.095	0.380	0.037	0.008	0.009
		Median	0.158	2.59	0.188	6.16	1.05	0.020	0.088	0.241	0.023	0.004	0.006
		Maximum	2.24	26.1	1.35	37.7	4.13	0.053	0.288	1.32	0.194	0.068	0.039
		Minimum	0.034	0.576	0.041	0.336	0.416	0.006	0.019	0.038	0.007	0.001	0.001

590 [a] Whisky samples additionally analysed by ICP spectroscopy.

591 Table 2

592 Comparison of TXRF sample preparation methods [a].

593

Element	Mean element concentration ± standard deviation (n = 10), mg L^{-1}	
	Pre-mixed	Mixed on disc
P	0.97 ± 0.11	0.96 ± 0.15
S	4.39 ± 0.24	4.21 ± 0.53
Cl	0.32 ± 0.10	0.25 ± 0.08
K	16.2 ± 0.59	16.2 ± 1.54
Ca	1.91 ± 0.08	1.91 ± 0.15
Mn	0.040 ± 0.003	0.041 ± 0.004
Fe	0.143 ± 0.025	0.139 ± 0.017
Cu	1.015 ± 0.029	0.999 ± 0.065
Zn	0.034 ± 0.004	0.031 ± 0.002
Br	0.007 ± 0.002	0.006 ± 0.002
Rb	0.013 ± 0.001	0.013 ± 0.002

594 [a] The tests above used whisky sample number 30, see Table 1.

595 Table 3

596 Limits of detection (LOD) for TXRF analysis results derived from the analysis of 32 whisky
597 samples before and after cold plasma ashing (CPA). The limits of detection for ICP analysis
598 are derived from the analysis of single element standards in 2 M nitric acid.
599

Element	Range		Mean		Median		ICP
	Original	CPA	Original	CPA	Original	CPA	
	mg L^{-1}						
P	0.084 - 0.367	0.073 - 0.338	0.142	0.134	0.120	0.116	0.020[a]
S	0.035 - 0.151	0.030 - 0.141	0.058	0.055	0.048	0.048	0.050[a]
Cl	0.026 - 0.112	0.022- 0.105	0.043	0.041	0.036	0.036	NA
K	0.009 - 0.042	0.008 - 0.039	0.016	0.015	0.013	0.013	0.040[a]
Ca	0.006 - 0.026	0.005 - 0.025	0.010	0.010	0.009	0.008	0.004a
Mn	0.002 - 0.007	0.001 - 0.007	0.003	0.003	0.003	0.002	0.001[b]
Fe	0.001 - 0.006	0.001 - 0.006	0.002	0.002	0.002	0.002	0.002[a]
Cu	0.001 - 0.004	0.001 - 0.003	0.001	0.001	0.001	0.001	0.004[a]
Zn	0.001 - 0.003	0.001 - 0.003	0.001	0.001	0.001	0.001	0.003[a]
Br	0.000 - 0.002	0.000 - 0.002	0.001	0.001	0.001	0.001	NA
Rb	0.000 - 0.003	0.000 - 0.002	0.001	0.001	0.001	0.001	0.001[b]

600 [a] ICP-OES; [b] ICP-MS. NA = Not available.
601

602 Table 4

603 Comparison of geometric means of element concentrations in whisky samples.

604

Whisky	Element (mg L^{-1})										
	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Br	Rb
Non counterfeit	0.222	2.04	0.235	8.46	0.997	0.023	0.083	0.347	0.026	0.004	0.007
SD[a] (n = 27)	0.622	2.34	0.249	10.7	0.729	0.012	0.060	0.326	0.035	0.006	0.010
Counterfeit	0.088	14.0	0.096	1.13	1.466	0.007	0.051	0.066	0.032	0.007	0.001
SD[a] (n = 5)	0.118	8.39	0.239	0.908	0.219	0.002	0.055	0.063	0.077	0.028	0.002

605 [a] Standard deviation.

606

List of Figures

Fig. 1. Example TXRF spectrum of whisky (sample number 30, Glenrothes, see Table 1).

Fig. 2. Comparison of element concentrations in thirty two whisky samples measured by TXRF before and after CPA.

Fig. 3. Comparison of element concentrations (mg L^{-1}) in eighteen sample of whisky measured by TXRF and ICP spectroscopy. The dotted line represents the 1:1 relationship.

Fig. 4. Principal components analysis score plots prepared from log transformed trace element concentration data for thirty two whisky samples

Fig. 5. Principal components analysis loadings plots prepared from log transformed trace element concentration data for thirty two whisky samples. For sample numbering and description of region or type, see Table 1.

Fig. 6. Dendogram showing correlation coefficient distances for the trace elements using standardised log transformed data. The numbers preceding the x-axis labels refer to the whisky sample numbers given in Table 1.

Fig. 7. Dendogram using Euclidean distance and standardised log transformed data showing linkages between trace element variables.

Supplementary information

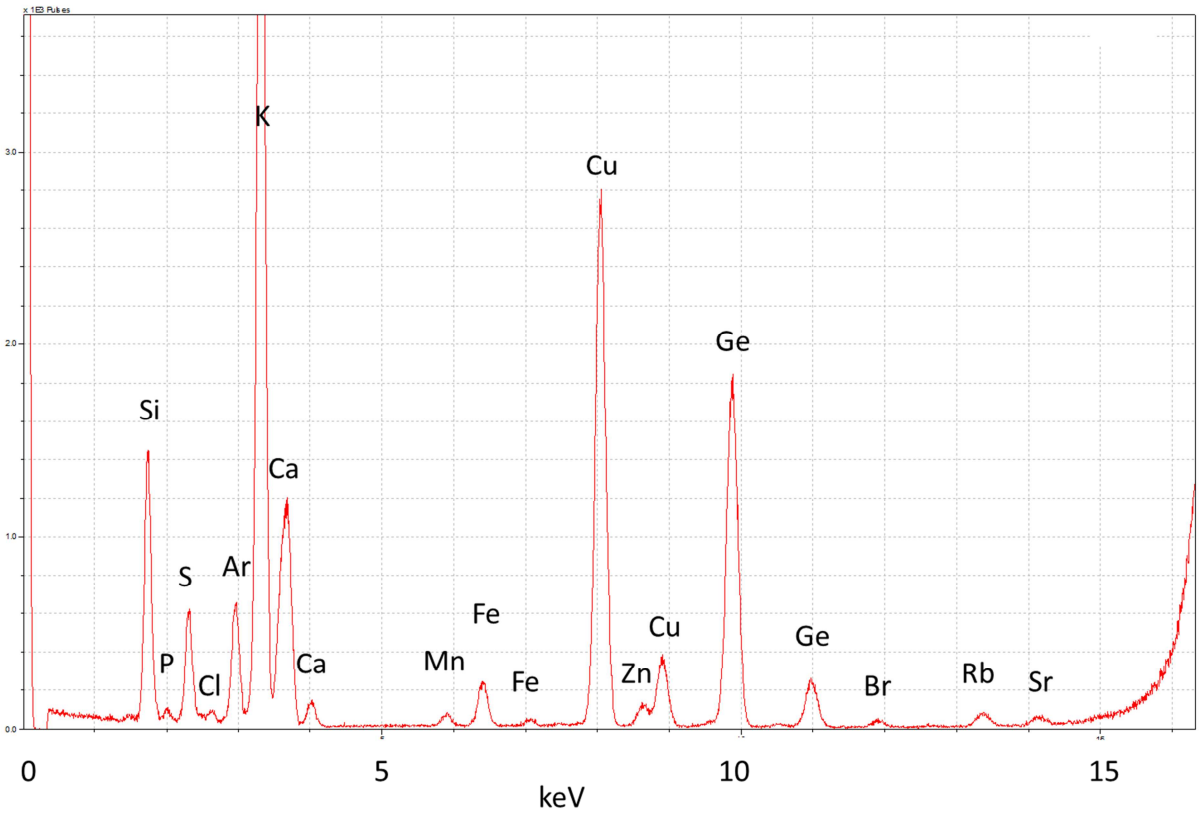
Fig. S1. Whisky producing regions of Scotland.

Fig. S2. FTIR spectra of whisky (sample number 21, Talisker, see Table 1) before and after oxygen plasma ashing.

639

640 Fig. 1

641



642
643

644 Fig. 2

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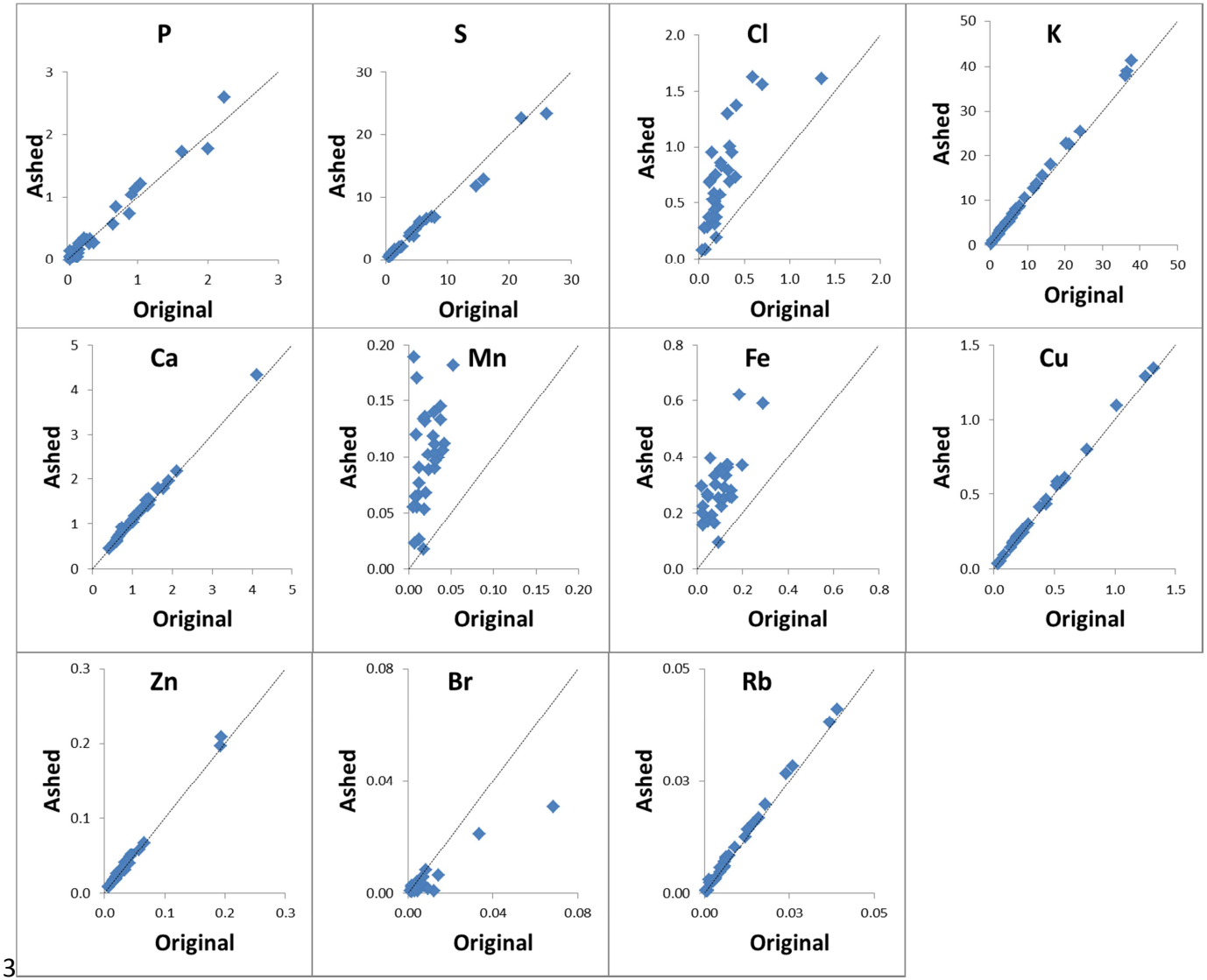
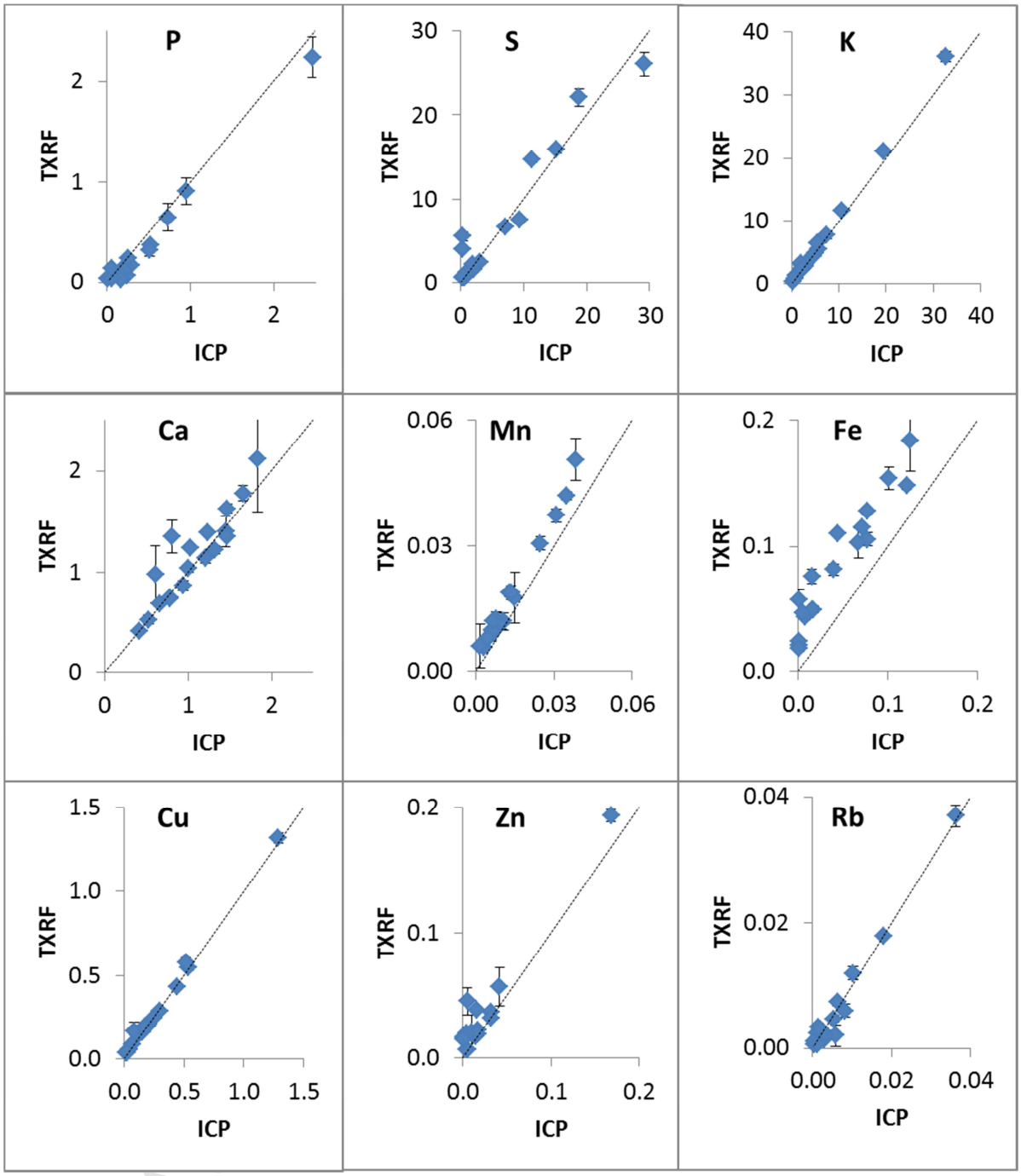


646 Fig. 3

647 Fig. 3

648



649

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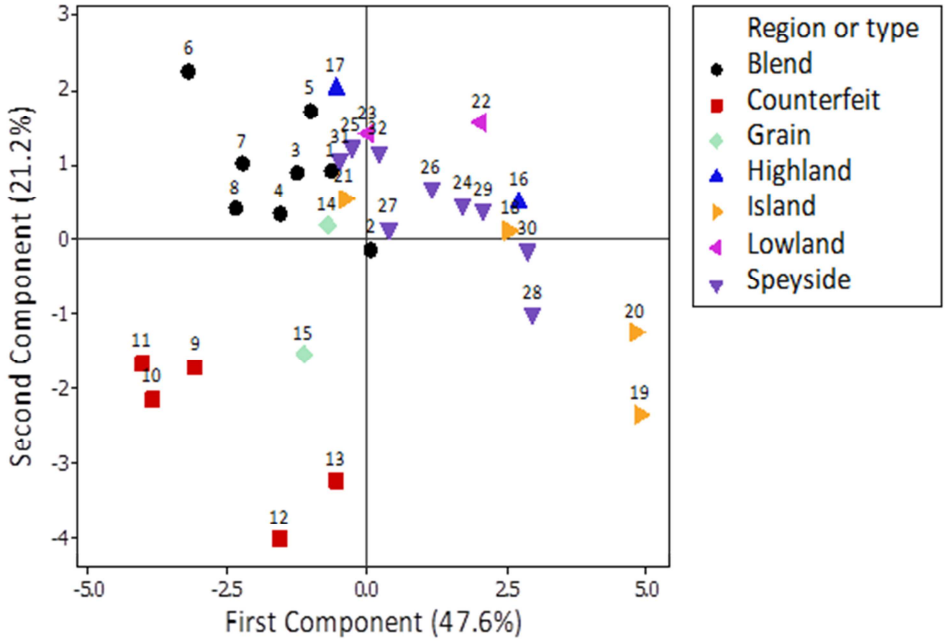
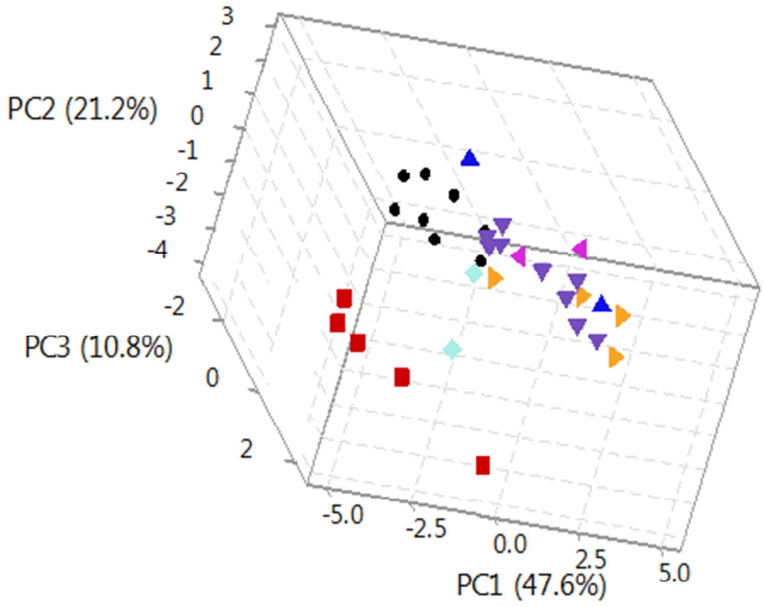
651 Fig. 4





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654 Fig. 5

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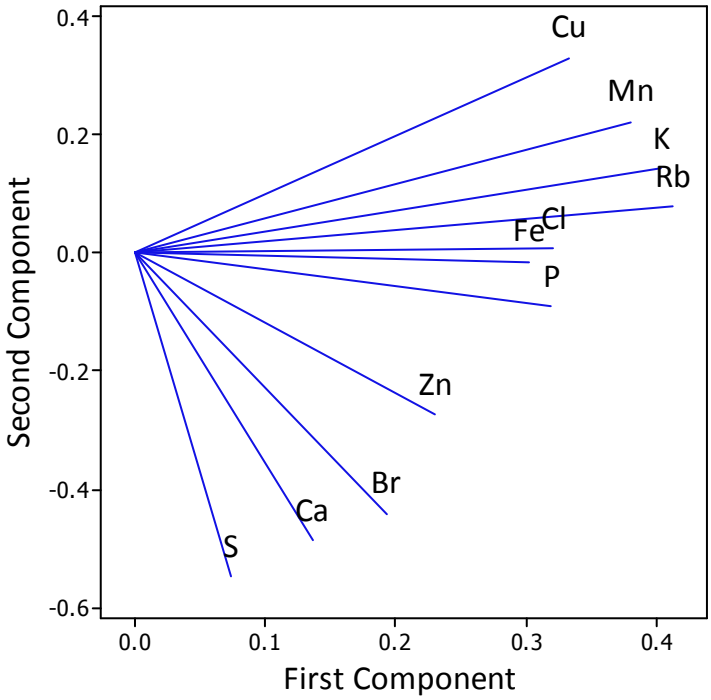

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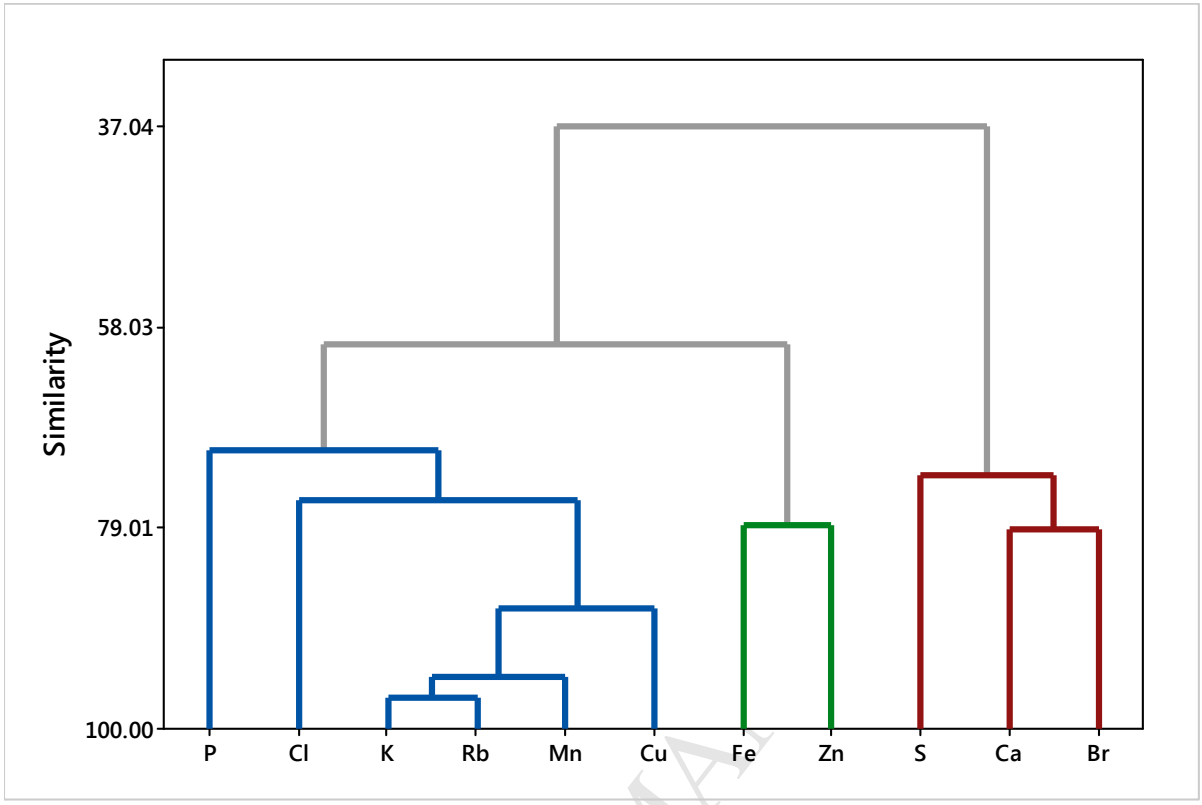
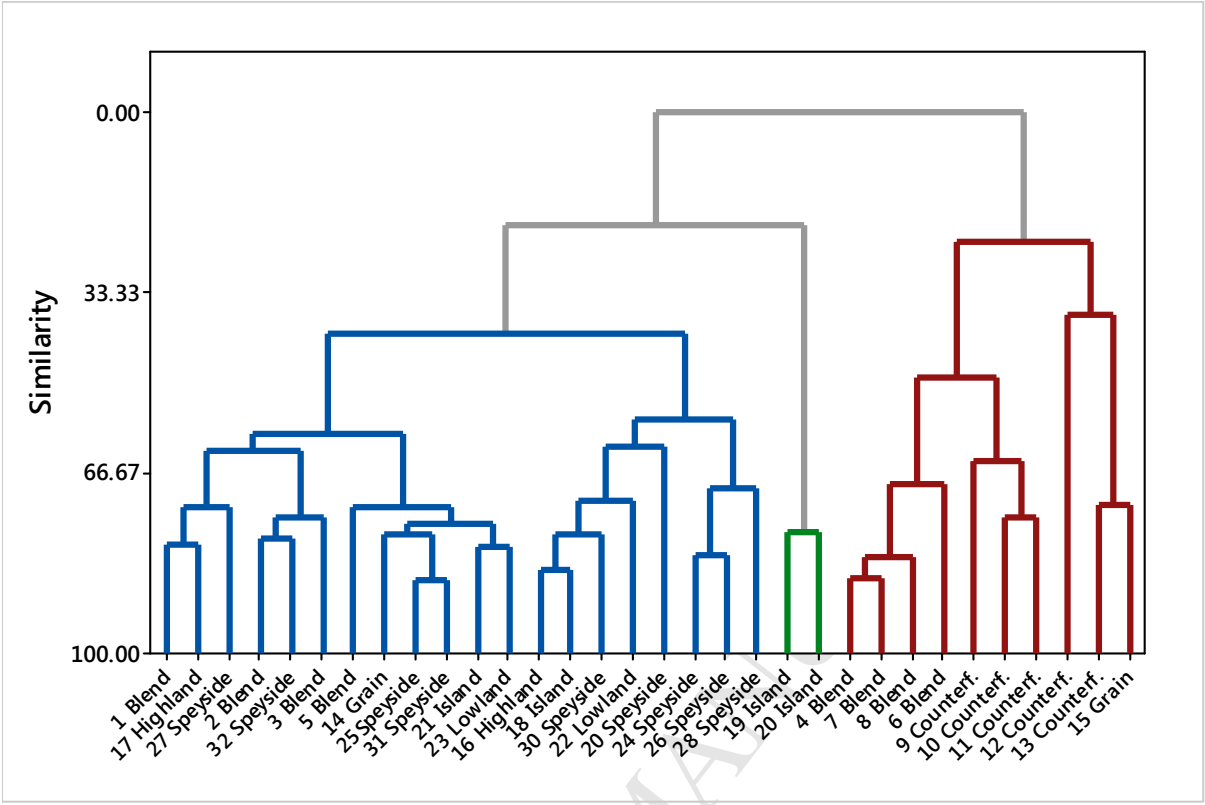
657 Fig. 6
658



659
660

661 Fig. 7
662



663
664

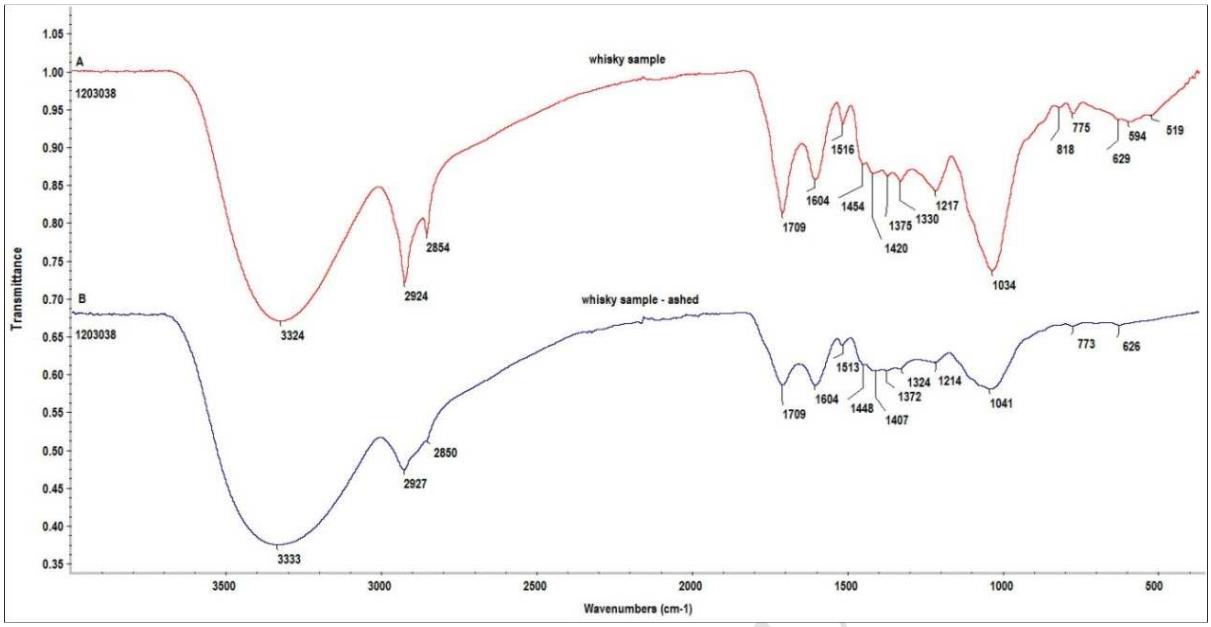
665 Fig. S1

666

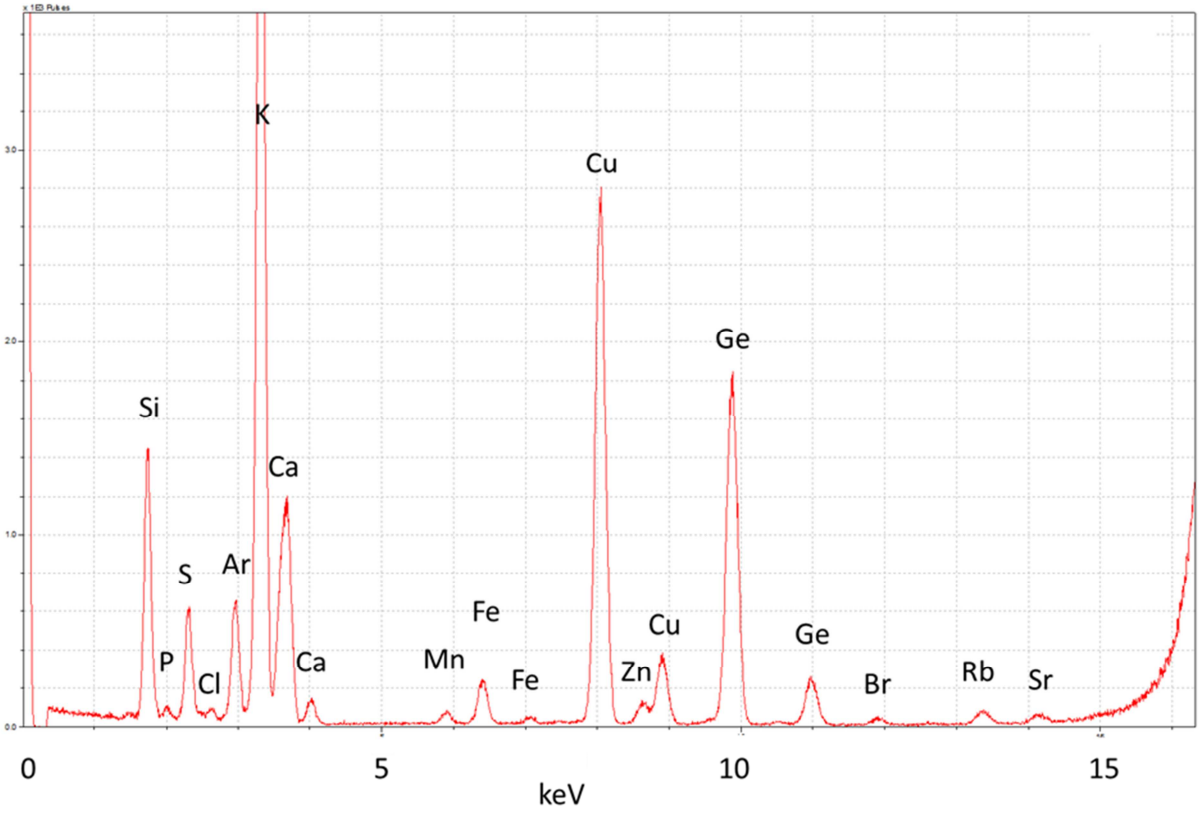
667

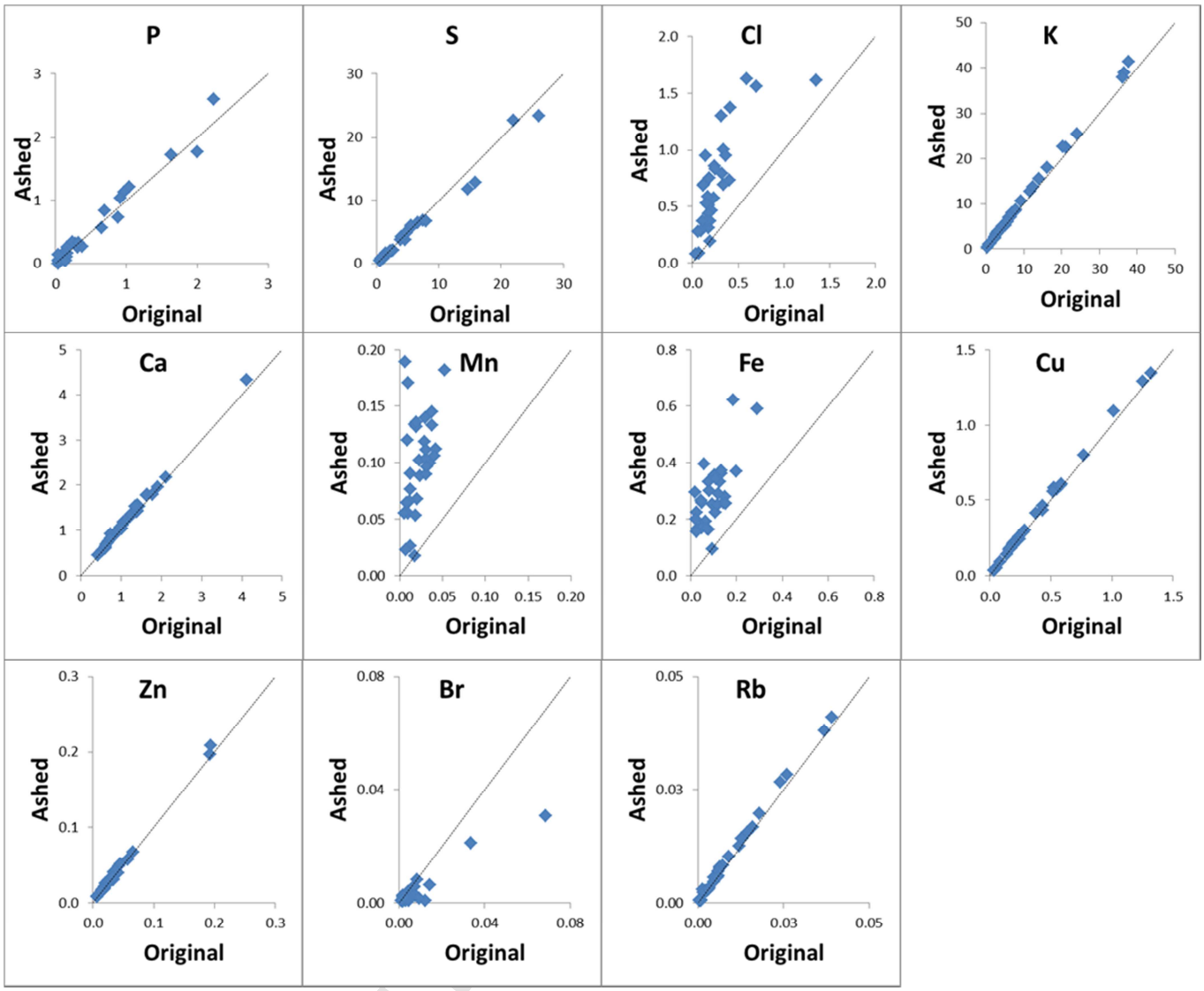
668 Fig. S2

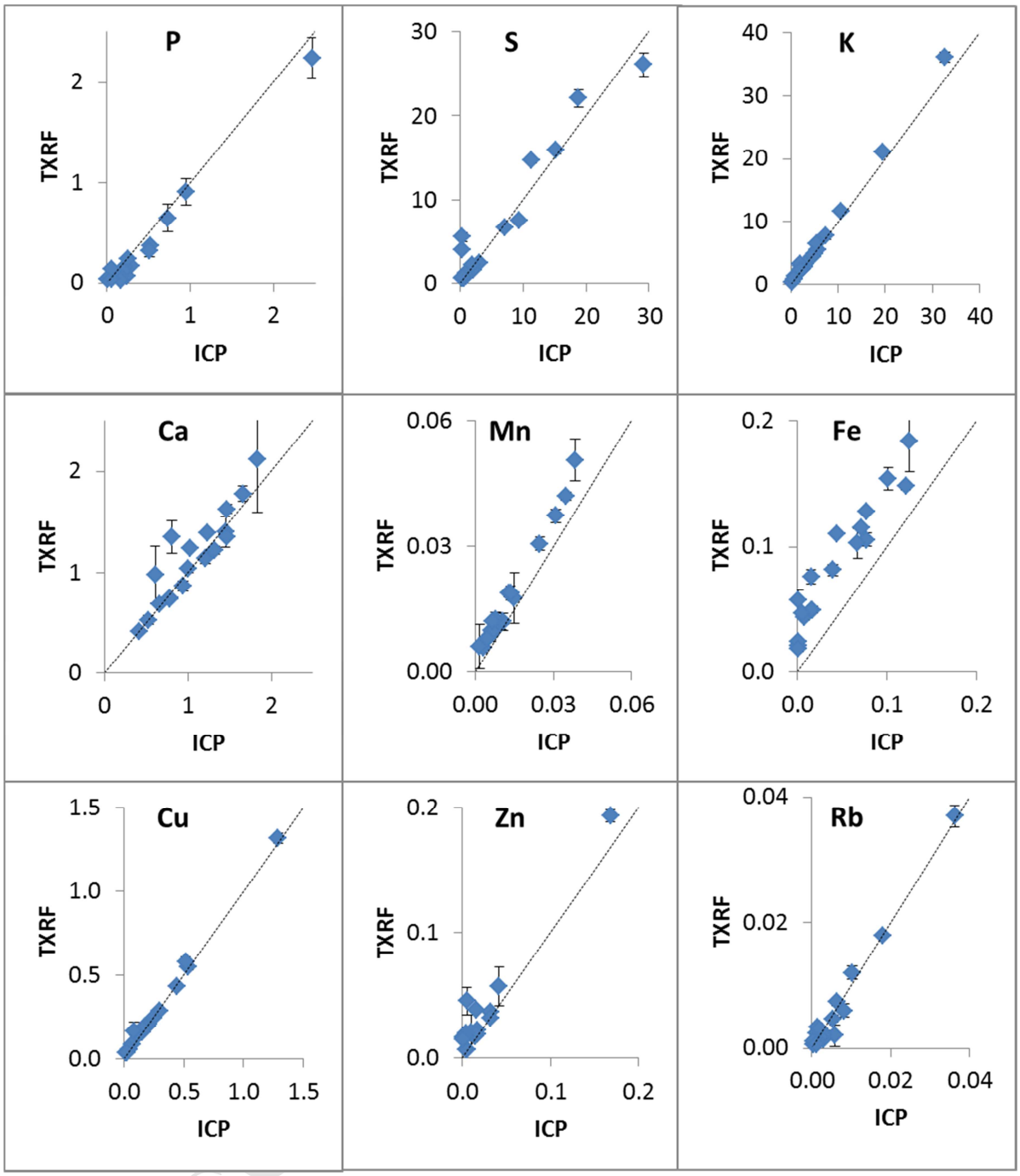
669

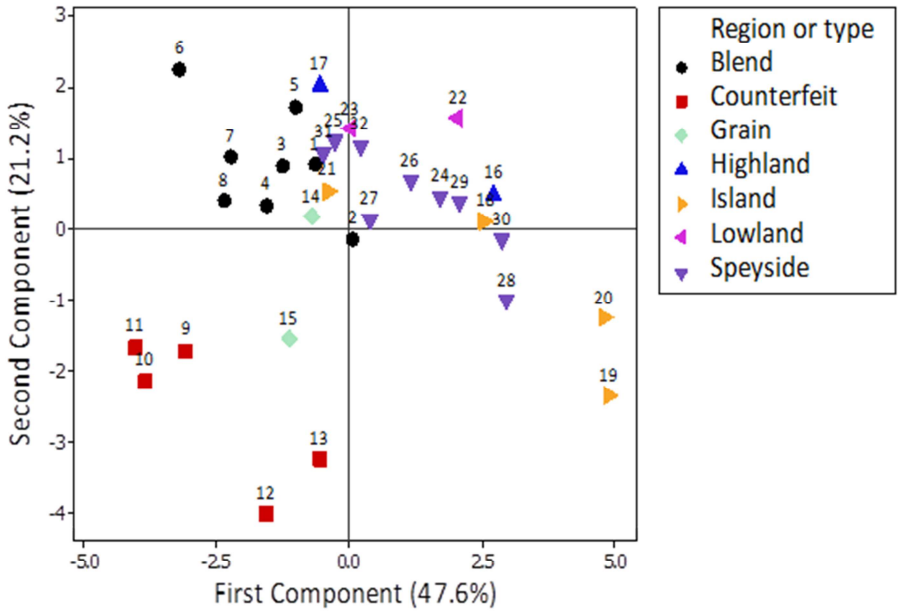


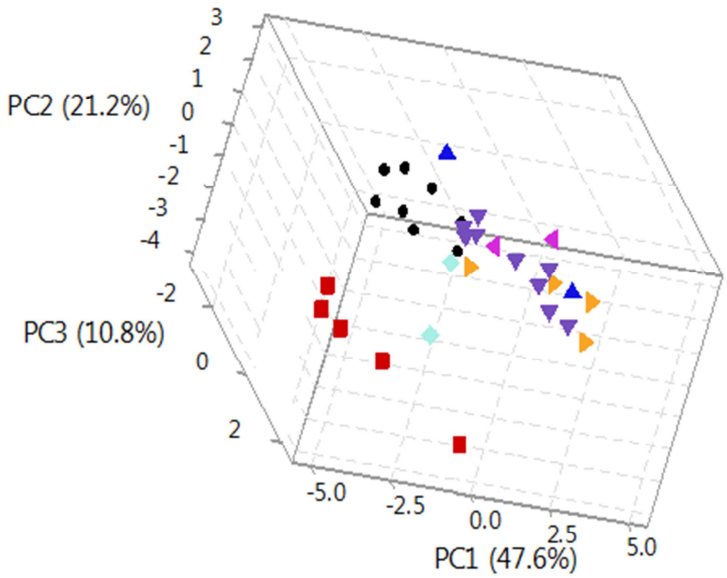
670

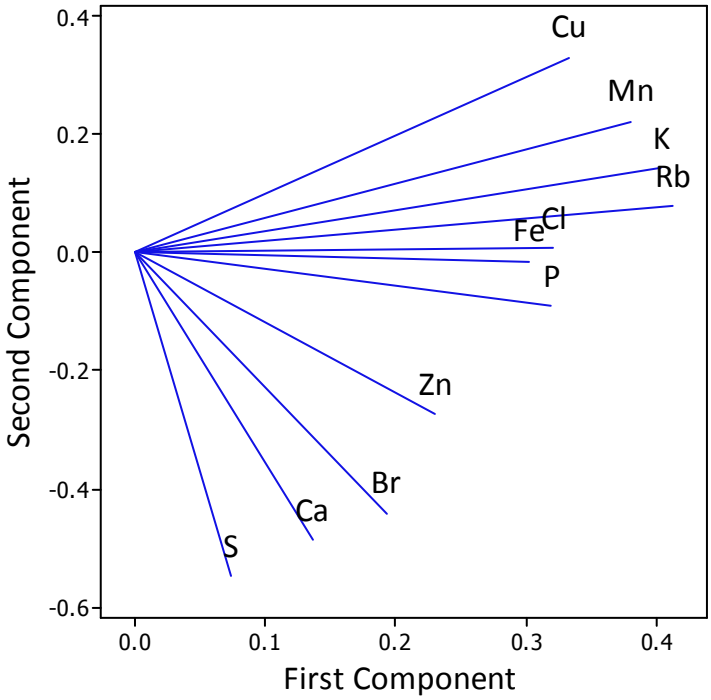


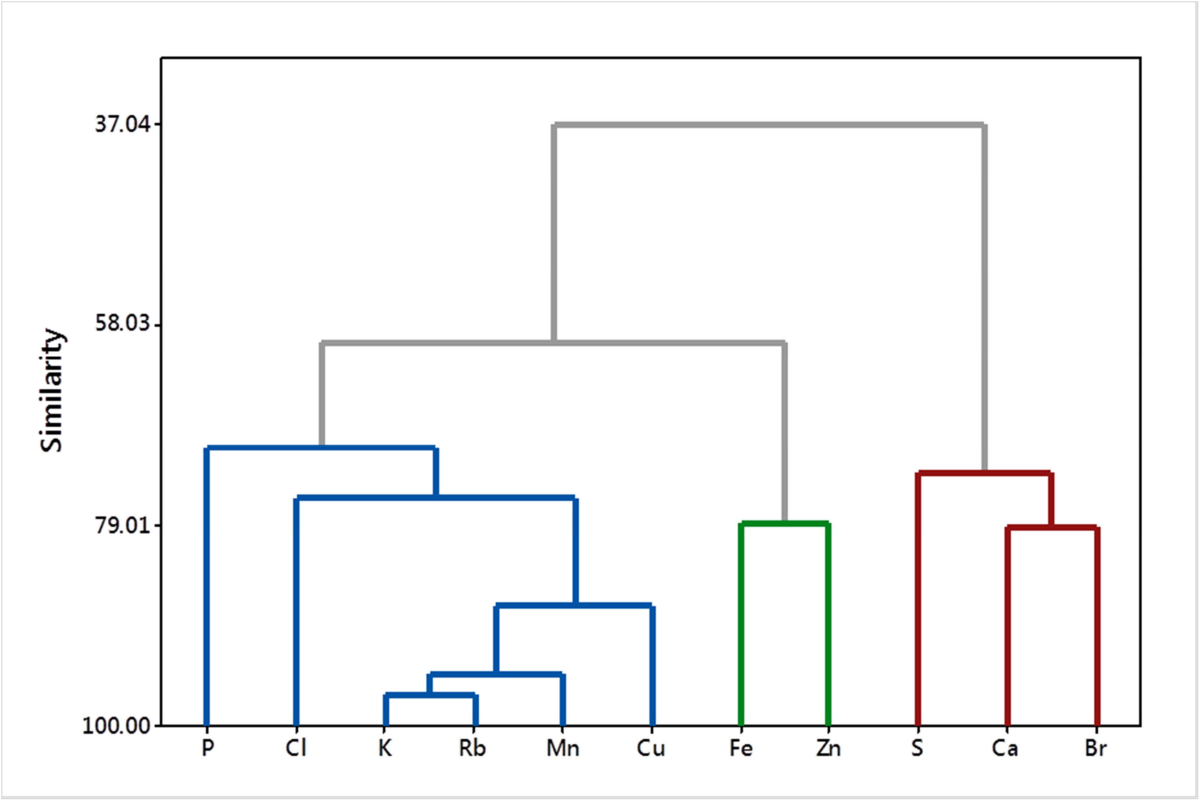


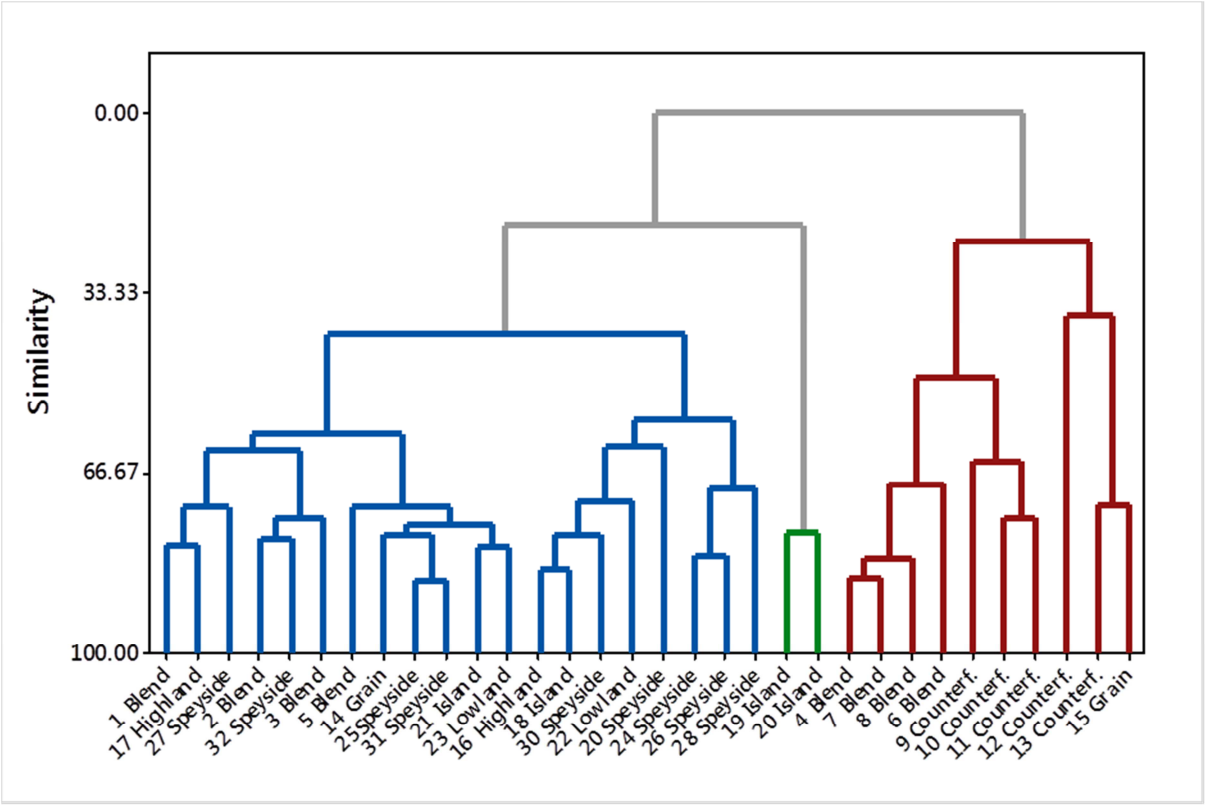

P
TXRF
ICP
0
1
2
S
TXRF
ICP
0
10
20
30
K
TXRF
ICP
0
10
20
30
40
Ca
TXRF
ICP
0
1
2
Mn
TXRF
ICP
0.00
0.03
0.06
Fe
TXRF
ICP
0.0
0.1
0.2
Cu
TXRF
ICP
0.0
0.5
1.0
1.5
Zn
TXRF
ICP
0.0
0.1
0.2
Rb
TXRF
ICP
0.00
0.02
0.04

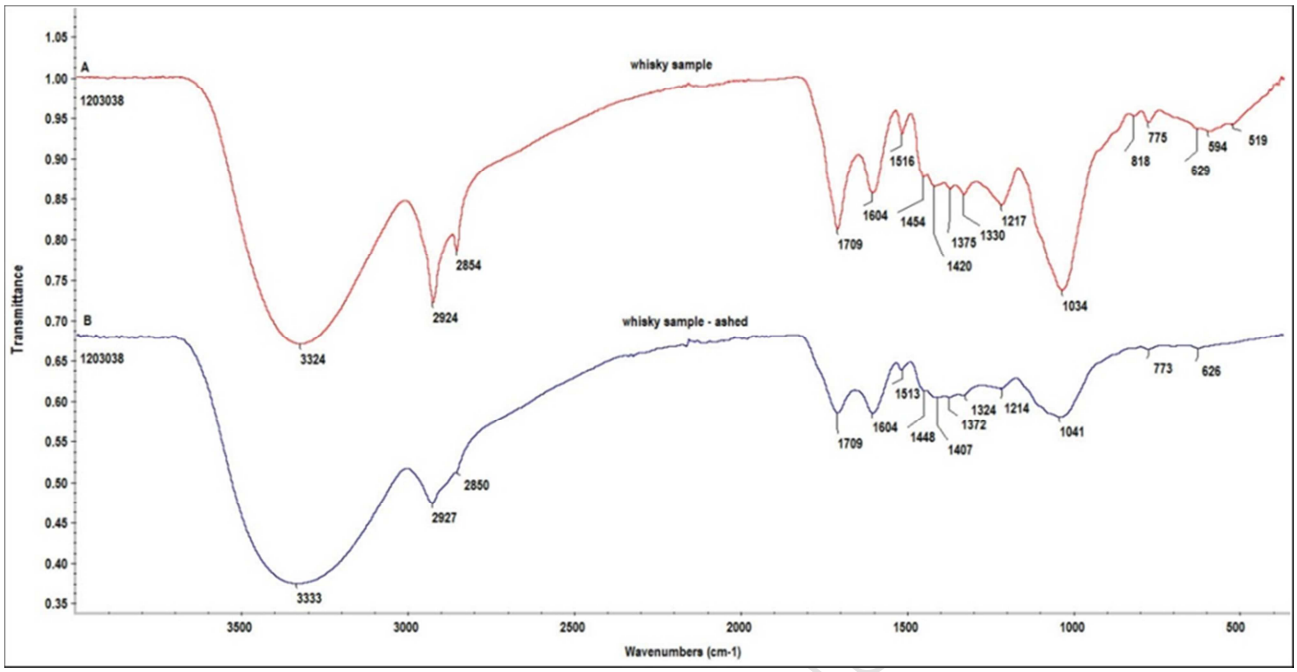

Region or type
Blend
Counterfeit
Grain
Highland
Island
Lowland
Speyside
Second Component (21.2%)
First Component (47.6%)


PC2 (21.2%)
PC3 (10.8%)
PC1 (47.6%)









Highlights

- TXRF was applied to the elemental analysis of Scotch whisky and counterfeits for 11 elements
- No sample pre-treatment was required apart from adding Ge as an internal standard
- TXRF limits of detection ranged from 0.1 to 0.001 mg L^{-1} and compared well with ICP
- Only a small volume of whisky sample was required (10 µL) for the TXRF analysis
- Statistical analysis of the data by PCA and LDA correctly classified the counterfeit whiskies

Dear Joel

The details (already supplied) are as follows. Is this not sufficient? I am at a loss to see what's missing!

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Thank you

Charlie