INVESTIGATING THE ORIGIN OF COTTON IN YARN USING MULTIVARIATE ISOTOPE PROFILES

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INTRODUCTION

The analysis of cotton fibres can be particularly challenging within a forensic science context where discrimination of one fibre from another is of importance. Normally cotton fibre analysis examines the morphological structure of the recovered material and compares this with that of a known fibre from a particular source of interest. Analysis of the dyes which may have been applied to the cotton fibre can also be undertaken though this can be difficult and unproductive in terms of discriminating one fibre from another.

The aim of this work was to explore the potential for Isotope Ratio Mass Spectroscopy (IRMS) to be utilised as an additional tool for cotton fibre analysis in an attempt to reveal further discriminatory information. For this proof-of-concept study we concentrated on un-dyed cotton fibres of known origin in order to expose the potential of the analytical technique.

Limited amounts of previous research have reported stable isotope analysis of cotton plants, leaves or cellulose and have investigated natural variation in ^{2}H and ^{18}O isotopic abundance in plant dry matter [1], carbohydrates [2] or the effect of water stress on ^{2}H, ^{18}O and ^{13}C isotopic abundance in cellulose [3].

However, these previous studies have confirmed the feasibility of using the ^{2}H, ^{18}O and ^{13}C isotopic composition of cotton cellulose present in cotton fabrics as a potential discriminatory tool. This can occur through the determination of the geographic provenance of the material because of the clear influence of the growth environment on the stable isotope profiles.

We report the results of a pilot study aimed at testing the hypothesis that multi-element stable isotope analysis of cotton fibres in conjunction with multivariate statistical analysis of the resulting isotopic abundance data permits sample provenancing based on the determination of where the cotton was grown and as such will facilitate sample discrimination [4].

Stable Isotope Background

What are Isotopes?

The basic building blocks of which we and everything around us are made, i.e. the natural chemical elements are listed in the Periodic Table of Chemical Elements in order of increasing atomic number Z, which is the same as number of protons in the nucleus of an atom. Should two atoms of the same chemical element (with same atomic number Z) contain a different number of neutrons N in their nuclei, they are called isotopes. Almost all of the 92 naturally occurring chemical elements are found in nature in more than one isotopic form. The vast majority of these isotopes are stable isotopes, which means they do not decay. Radioisotopes by contrast are not stable and hence, undergo radioactive decay during which the parent element is transformed into a lighter daughter element with a lower atomic number than the parent element. In this context “almost all” means with the exception of 21 elements such as fluorine and phosphorous, which are mono-isotopic.

The feature that characterises a chemical element, that defines its chemical nature and makes e.g. carbon behave differently from sulphur is the number of protons in its nucleus since this is matched by the number of electrons surrounding the nucleus. The number of electrons and their quantum mechanical status define the nature and number of bonds a chemical element can form. A hydrogen atom contains one proton and one electron, a carbon atom contains six protons and six electrons. The most abundant kind of carbon atom also contains six neutrons and this carbon species is called carbon-12 (^{12}C) since its atomic mass is 12 atomic mass units or 12 amu. However, there is a less abundant form of carbon whose nucleus contains still six protons but seven neutrons. Since the number of protons and, hence number of electrons in this atom have not changed, the chemical characteristics of this atom are still those of carbon. However, since the additional neutron in its nucleus has increased its atomic weight by 1 atomic mass unit [amu] from 12 amu to 13 amu, it is called carbon-13 (^{13}C). So, in summary, isotopes of a given element contain the same number of protons (and electrons) and hence share the same chemical characteristics but they contain different numbers of neutrons and are therefore of different atomic mass.

Stable Isotope Abundance and the δ-Notation

Typically global or mean figures for isotope abundances of an element are given as atom percentages (see Table 1). For ^{12}C and ^{13}C the figures quoted in textbooks are 98.89 atom% and 1.11 atom%, respectively. In the case of sulphur the mean isotope abundance figures for ^{32}S, ^{34}S and ^{36}S are 95.02 atom%, 4.22 atom% and 0.76 atom%, respectively. However, by using the above global isotope abundance figures one looses sight of the variation in natural abundance of any given element isotope. For ^{13}C, the range of this natural abundance variation is of the order of 0.11 atom% [5]. Approximately 75% or 0.0824 atom% of this variation range falls into the interval from 1.0343 to 1.1167 atom%. Changes in isotope abundance at natural abundance level cannot be reliably detected, let alone measured and quantified by analytical instruments routinely used in an analytical chemistry laboratory. Scanning

organic desk-top mass spectrometers (MS) equipped with a single electron multiplier detector provide only an approximation of isotopic abundance through detection of isotope satellite ions, e.g. $M+1^+$ and comparing its selected ion current with that for the corresponding major abundant fragment ion M^+ [6]. Staying with the example of carbon and its minor isotope ^{13}C, in day-to-day practice scanning desktop MS systems can reliably detect changes in isotope abundance only if a compound has been labelled with ^{13}C so the ^{13}C content of the compound is in excess of 0.5 atom% above the typical natural abundance level of 1.11 atom%, i.e. containing > 1.61 atom% of ^{13}C.

Given that isotope abundance is measured by instruments with dedicated channels per isotope (multi-collector MS), expressing natural abundances of stable isotopes in a given material as the ratio of the minor (usually heavier) over the major abundant (usually lighter) isotope of a given element (e.g. $^2H/^1H$ or $^{13}C/^{12}C$) has been adopted as the preferred notation. Since changes in isotope composition of light elements at natural abundance level are relatively minute and isotope abundances of the minor isotopes are very small, significant changes when given in atom% happen typically in the 2nd decimal place. Similarly, changes in the ratio of heavier : lighter isotope are usually confined to the numerals in the 3rd and 4th decimal places. Due to the minute nature of these changes in isotope abundance at natural abundance level, measured isotope ratios of a given material are expressed relative to a contemporaneously measured isotope ratio of a standard of known isotopic composition. To make the resulting figures more manageable the "delta notation"(δ) was adopted. The δ-value of the heavier isotope h of a chemical element E in a sample is defined by the following equation (equation 1) [5, 7] where R_{sample} is the measured isotope ratio of the heavier isotope over the lighter (e.g. $^{13}C/^{12}C$) for the sample and $R_{Standard}$ is the measured isotope ratio for the corresponding international reference material (e.g. Vienna Pee Dee Belemnite [VPDB] in the case of ^{13}C).

$$\delta^{h}E = [(R_{sample} / R_{standard}) - 1] \times 1000 \qquad \text{Equation 1}$$

The results of this equation are expressed in ‰ (per mil). A positive δ value is interpreted to mean the sample has a higher abundance of the heavier isotope than the international reference standard that defines the scale for a particular isotope, while a negative δ value is interpreted to mean the sample has a lower abundance of the heavier isotope than the international reference standard. By virtue of its definition, the δ value of a scale defining international reference standard is 0 ‰. International standards that define the scale of a particular isotope are called Calibration Materials (CMs), while international standards whose isotopic composition have been certified by direct comparison with the relevant CM are called Reference Materials (RMs). CMs and RMs are certified and administered through the International Atomic Energy Agency (IAEA) in Vienna, Austria, or the National Institute of Standards and Technology (NIST), Gaithersburg, USA.

Materials and Methods

Sample preparation

Known provenance un-dyed cotton samples were obtained from a United Kingdom cotton fibre supplier. The supplied samples originated from Egypt (2 samples), Turkey, Argentina and Uzbekistan. Each of the cotton samples consisted of spun un-dyed fibres. Five sub-samples were taken at random from different areas of each sample fibre to obtain representative sample mean values thus accounting for potential sample inhomogeneity.

Given that the objective of this study was to determine whether or not differences in cotton provenance were reflected in significant relative differences in stable isotopic composition, samples were not subjected to a two-stage hydrogen equilibration procedure required for determination of true δ^2H-values [8-10]. The molar exchange fraction F(ex) of exchangeable hydrogen in cellulose was determined to be 0.027 and resulting changes in δ^2H values if corrected for this value of F(ex) would have been of the same order as their associated measurement uncertainty. Following the principle of identical treatment[11], all samples were equilibrated for seven days in a sealed desiccator over water of known isotopic composition before being transferred into a drying-down desiccator prepared with self-indicating, non-caking phosphorus pentoxide as drying agent (Sicapent, Merck Chemicals, UK). Once weighed out into either silver or tin capsules for ^{2}H/^{18}O or ^{13}C/^{15}N isotope analysis, respectively, samples were dried down under vacuum in a desiccator over Sicapent for 7 days. Dried down samples were immediately loaded into a solid Costech Zero-Blank autosampler (Pelican Scientific Ltd, Alford, UK), which was purged with helium and sealed to avoid any re-introduction of moisture from ambient humidity[12].

Stable isotope analysis

Bulk ^{13}C / ^{15}N isotope analysis

Samples were weighed into tin capsules so each capsule contained typically 0.5 mg of sample and were analysed for their ^{15}N and ^{13}C isotopic composition using an automated nitrogen-carbon analyser (ANCA) coupled to a 20/20 isotope ratio mass spectrometer (IRMS) (SerCon Ltd, Crewe, UK). The Elemental Analyzer (EA) reactor tubes were comprised of two quartz glass tubes filled with chromium(III) oxide / copper oxide and reduced copper, held at 1020℃ and 620℃ for combustion and reduction, respectively. A water trap filled with magnesium perchlorate was used to remove water from generated combustion gases and a post-reactor GC column was kept at 65℃ for separation of evolved N_2 and CO_2. Data were processed using proprietary software (SerCon Ltd, Crewe, UK).

Two international reference materials were used for scale calibration; IAEA-CH6 (δ^{13}C = -10.45‰, IAEA, Vienna, Austria) and IAEA-600 (δ^{13}C = -27.77‰, δ^{15}N = 1.00‰, IAEA, Vienna, Austria). Two different analytical quality control samples (AQCs) were also analysed with each batch for quality control purposes. These two AQCs were glutamic acid (δ^{15}N: -5.04 ‰ and δ^{13}C: -28.50 ‰) and leucine (δ^{15}N:

10.77‰ and δ^{13}C: -31.18‰). Analytical error of the instrument for measured δ^{13}C and δ^{15}N values was calculated to be ± 0.16‰ and ± 0.20‰, respectively based on measurements of AQCs and reference materials. Typical standard deviation for 5 replicates of each sample was 0.38‰ for δ^{13}C and 2.32‰ for δ^{15}N, with the latter being a reflection of the samples' low relative N abundance of <0.3 %N.

Bulk ^{2}H / ^{18}O isotope analysis

A Delta^{Plus}-XP isotope ratio mass spectrometer (IRMS) coupled to a high temperature conversion / elemental analyzer (TC/EA; both Thermo-Fisher Corporation, Bremen, Germany) was used for ^{2}H/^{1}H and ^{18}O/^{16}O isotope ratio measurement. Typically, 0.2 mg of a sample was weighed into a silver capsule and placed in a desiccator for one week before the samples were introduced into the TC/EA by means of a Costech Zero-Blank solid autosampler (Pelican Scientific Ltd, Alford, UK). The reactor tube was self-packed and comprised of an Alsint^{TM} ceramic tube, which contained a glassy carbon tube filled with glassy carbon granulate, silver and quartz wool (SerCon, Crewe, Cheshire). The reactor temperature was set to 1425℃ while the post-reactor GC column was maintained at 85℃. Data were process ed using proprietary Isodat NT software, version 2.0 (Thermo-Fisher Corporation, Bremen, Germany).

Two international reference materials and two in-house standards were used for scale calibration to VSMOW; IAEA-CH7 (δ^{2}H = -100.3 ‰, IAEA, Vienna, Austria), IAEA-602 (d^{18}O = 71.4 ‰, IAEA, Vienna, Austria), coumarin (δ^{2}H = 62.6 ‰, d^{18}O = 15.83, Iso-Analytical, Crewe UK) and glucose penta-acetate (δ^{2}H = -98.5 ‰, d^{18}O = 23.94 ‰, Iso-Analytical, Crewe, UK). Analytical error of the instrument for measured δ^{2}H and δ^{18}O values was calculated to be ± 1.0 ‰ and ± 0.15 ‰, respectively based on replicate measurements of the reference materials. Standard deviation for 5 replicate analyses of each sample ranged from 0.4 to 1.9 ‰ and 0.1 to 0.3 ‰ for δ^{2}H and δ^{18}O values, respectively.

Results and Discussion

Water taken up through their root system is the sole hydrogen precursor pool available to plants for the biosynthesis of plant products such as starch or cellulose. How closely the ^{2}H isotopic composition of a particular plant product will reflect that of source water depends on the chemical nature of the plant product (e.g. lipid vs. carbohydrate) and the varying degrees of isotopic fractionation associated with the different biosynthetic pathways. In addition, potential H exchange between cell water and intermediate as well as final plant product, which again varies depending on the chemical nature of the compounds made, has an influence on the ^{2}H isotopic composition of the final plant product. For these reasons, observed δ^{2}H values for different plant products within the same plant can range from -200 to +20‰ with δ^{2}H values for carbohydrates such as sugars and cellulose showing a small net fractionation, i.e. being numerically close to corresponding δ^{2}H values for source water[5, 10]. With this in mind, δ^{2}H values of cotton should be a good selector (a) to distinguish between cotton of different geographic origin and (b) to determine sample provenance.

Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) was applied to the isotopic data derived from analysis of the samples. In line with the aforementioned considerations, only data relating to hydrogen and oxygen data produced results of any significance in terms of sample discrimination.

Looking at the VSMOW scale corrected δ^2H values observed for the different cotton samples (Table 1), a first glance reveals four groups of results clustering around -39.5, -24.6, -8.2 and +13.5‰ on average. On the basis of VSMOW scale corrected δ^{18}O values only two groups of results seemed to emerge clustering around 29 and 33‰. Interestingly enough, the cluster around δ^{18}O values of 33‰ is comprised of the same three samples showing the highest (most positive) δ^2H values observed ranging from -8.2 to +15.2‰ (Figure 1). This tentative grouping of Egyptian cotton samples into one group based on their ^{2}H and ^{18}O isotopic composition was supported by principal component analysis (PCA) shown in Figure 2. HCA analysis on the other hand placed the Egyptian cotton samples into two different clusters that were however well resolved from all other sample groups. HCA also suggested a linkage between the Turkish and Argentinean samples, which PCA resolved into two separate groups (Figure 3). Both PCA and HCA placed the Uzbekistan cotton samples into one group distinctly different from all other sample groups.

According to data collated by the Global Network of Isotopes in Precipitation (GNIP) the ^{2}H isotopic composition of meteoric water in Egypt ranges from -38 to -14‰ [http://www.iaea.org/water]. Taking the average δ^2H value of all three Egyptian cotton samples (= 6.3‰) and determining its net isotopic shift from mean source water (= -26‰), one arrives at a difference of 30.3‰. This, of course, makes the simplifying assumption that all three batches of cotton were grown and harvested under similar, if not the same conditions such as geographic area, soil type, temperature, water and nutrient supply.

At the other end of the scale of observed δ^2H values with a mean δ^2H value of -39.5‰ was the sample of cotton grown in Uzbekistan. Again, this sample was clearly separated from all other samples by both stable isotope data plot (Figure 1), PCA (Figure 2) and HCA (Figure 3). The GNIP database puts the ^{2}H isotopic composition of meteoric water in Uzbekistan in a range from -70 to -38‰. If one takes the arithmetic mean of this range (= -54‰) and compares it to the δ^2H value of -39.5‰ obtained for the cotton sample from Uzbekistan, one ends up with a net isotopic shift of 14.5‰.

Applying the same principle to the samples from Argentina (GNIP: -38 to -30‰) and Turkey (GNIP: -54 to -30‰) we arrived at net isotopic shift values of 10 and 1 ‰, respectively. The pooled shift value observed for the Egyptian samples was approximately twice that observed for all other samples. This was most likely due to differences in the growth cycles of the plants where sample BGF112 with a net isotopic shift calculated as 17.8‰, (which was in good agreement with the corresponding shift values for cotton from Argentina, Turkey and Uzbekistan) was possible grown in a year with moderate temperature changes resulting in little evaporative loss of river water and, hence only small changes in its isotopic

composition. The other two Egyptian cotton samples demonstrated somewhat higher net isotopic shift values of 37.8‰ (sample BZY112A) and 41.2‰, (sample BZY112B) 41.2‰ and were probably grown in similar environmental conditions when temperatures were high resulting in substantial evaporative loss of the water running through irrigation channels and, hence resulting in large changes in the isotopic composition of the remaining water, which due to the preferential loss of isotopically light water[7] will become more and more enriched in ^{2}H.

CONCLUSIONS

This short study has demonstrated for the first time the potential for isotopic analysis to be used as an aid to discriminate natural fibres from each other or to elucidate sample history or provenance based on geographical origin [4]. This may have potential uses in the forensic analysis of cotton fibres in circumstances when such discrimination is required, e.g. in cases when information on cotton provenance may provide vital investigative focus. However, in order to draw meaningful (forensic) conclusions from ^{2}H, ^{13}C and ^{18}O isotope data of cotton, a much larger sample set of cotton grown in these countries would have to be collected and analysed to obtain a robust statistical measure of their natural range and variance on which a set of exclusion criteria could be based.

Reference List

[1] Gan, K.S., Wong, S.C., Yong, J.W.H. & Farquhar, G.D. (2002). O-18 spatial patterns of vein xylem water, leaf water, and dry matter in cotton leaves, *Plant Physiology* **130**(2), 1008-1021.

[2] Yakir, D. (1992). Variations in the Natural Abundance of O-18 and Deuterium in Plant Carbohydrates, *Plant Cell And Environment* **15**(9), 1005-1020.

[3] Yakir, D., Deniro, M.J. & Ephrath, J.E. (1990). Effects of Water-Stress on Oxygen, Hydrogen and Carbon Isotope Ratios in 2 Species of Cotton Plants, *Plant Cell And Environment* **13**(9), 949-955.

[4] Daeid, N.N., Meier-Augenstein, W. & Kemp, H.F. (2011). Investigating the provenance of un-dyed spun cotton fibre using multi-isotope profiles and chemometric analysis, *Rapid Communications in Mass Spectrometry* **25**(13), 1812-1816.

[5] Fry, B. (2006). *Stable Isotope Ecology*, Springer, New York, NY 10013, USA.

[6] NicDaeid, N. & Meier-Augenstein, W. (2008). Feasibility of source identification of seized street drug samples by exploiting differences in isotopic composition at natural abundance level by GC/MS as compared to isotope ratio mass spectrometry (IRMS), *Forensic Science International* **174**(2-3), 259-261.

[7] Sharp, Z.D. (2007). *Principles of Stable Isotope Geochemistry*, Pearson Prentice Hall, Upper Saddle River, NJ 07458.

[8] Bowen, G.J., Chesson, L., Nielson, K., Cerling, T.E. & Ehleringer, J.R. (2005). Treatment methods for the determination of delta H-2 and delta O-18 of hair keratin by continuous-flow isotope-ratio mass spectrometry, *Rapid Communications in Mass Spectrometry* **19**(17), 2371-2378.

[9] Fraser, I. & Meier-Augenstein, W. (2007). Stable ^{2}H isotope analysis of human hair and nails can aid forensic human identification, *Rapid Communications in Mass Spectrometry* **21**(20), 3279-3285.

[10] Meier-Augenstein, W. (2010). *Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis*, John Wiley & Sons Ltd., Chichester, UK.

[11] Werner, R.A. & Brand, W.A. (2001). Referencing strategies and techniques in stable isotope ratio analysis, *Rapid Communications in Mass Spectrometry* **15**(7), 501-519.

[12] Landwehr, J.M., Meier-Augenstein, W. & Kemp, H.F. (2011). A counter-intuitive approach to calculating non-exchangeable 2H isotopic composition of hair: treating the molar exchange fraction fE as a process-related rather than compound-specific variable, *Rapid Commun. Mass Spectrom.* **25**(2), 301-306.

Table 1: Individual and average δ^2H, δ^{18}O and δ^{13}C values for cotton from different countries of origin.

Sample ID	δ^2H vs. VSMOW			δ^{18}O vs. VSMOW			δ^{13}C vs. VPDB			Country
		Average	S.D.		Average	S.D.		Average	S.D.	Batch No.
YDC001-1	-28.2			29.22			-24.10			
YDC001-2	-25.9			29.48			-23.94			
YDC001-3	-26.7			29.29			-24.23			
YDC001-4	-23.4			29.28			-23.49			Turkey
YDC001-5	-24.1	-25.0	1.5	29.47	29.38	0.11	-24.35	-24.02	0.33	8k03010
BZY112A-1	9.7			33.07			-24.00			
BZY112A-2	11.8			33.43			-24.03			
BZY112A-3	12.5			33.46			-23.45			
BZY112A-4	11.0			33.09			-24.29			Egypt
BZY112A-5	11.9	11.8	0.6	33.13	33.24	0.20	-23.61	-23.88	0.34	1008
BQF112-1	-7.4			31.78			-23.46			
BQF112-2	-6.9			31.80			-22.86			
BQF112-3	-9.7			31.37			-22.69			
BQF112-4	-9.5			31.31			-23.25			Egypt
BQF112-5	-7.3	-8.2	1.3	31.98	31.65	0.29	-22.99	-23.05	0.31	8B950tpm
BZY112B-1	15.3			33.73			-23.96			
BZY112B-2	15.6			33.85			-23.68			
BZY112B-3	15.5			33.77			-24.21			
BZY112B-4	15.4			34.07			-24.23			Egypt
BZY112B-5	14.5	15.2	0.4	33.73	33.83	0.14	-24.18	-24.05	0.23	9001B
RJX023A-1	-22.5			29.16			-23.47			
RJX023A-2	-21.7			29.29			-23.99			
RJX023A-3	-24.9			28.98			-23.11			
RJX023A-4	-25.1			28.98			-23.14			Argentina
RJX023A-5	-22.7	-23.4	1.5	29.30	29.14	0.16	-23.43	-23.43	0.35	1212B
RJX023B-1	-26.0			28.42			-23.03			
RJX023B-2	-24.8			28.87			-23.38			
RJX023B-3	-25.1			28.88			-22.34			
RJX023B-4	-24.9			28.85			-23.20			Argentina
RJX023B-5	-25.8	-25.3	0.6	28.73	28.75	0.19	-23.22	-23.03	0.41	1206B
AAH023-1	-39.4			28.64			-22.32			
AAH023-2	-41.2			28.95			-22.83			
AAH023-3	-38.1			29.04			-23.05			
AAH023-4	-39.5			29.24			-23.06			Uzbekistan
AAH023-5	-39.5	-39.5	1.1	29.25	29.03	0.25	-23.08	-22.87	0.32	27-22-01

Figure Legends

Figure 1: 3D plot of δ^2H, δ^{18}O and δ^{13}C centroid values of cotton from different geographic locations. Sample prefix B, Y, R and A denote Egyptian, Turkish, Argentine and Uzbek origin, respectively.

Figure 2 : PCA analysis of hydrogen and oxygen isotopic data demonstrating the grouping of samples by region of origin. 1 = Turkey; 2 = Egypt A1; 3 = Egypt A2 ; 4= Egypt B; 5 = Argentina A1; 6 = Argentina A2; 7 = Uzbekistan

Figure 3 : HCA analysis of hydrogen and oxygen isotopic data using correlation coefficient distances and single linkage.

Figure 1

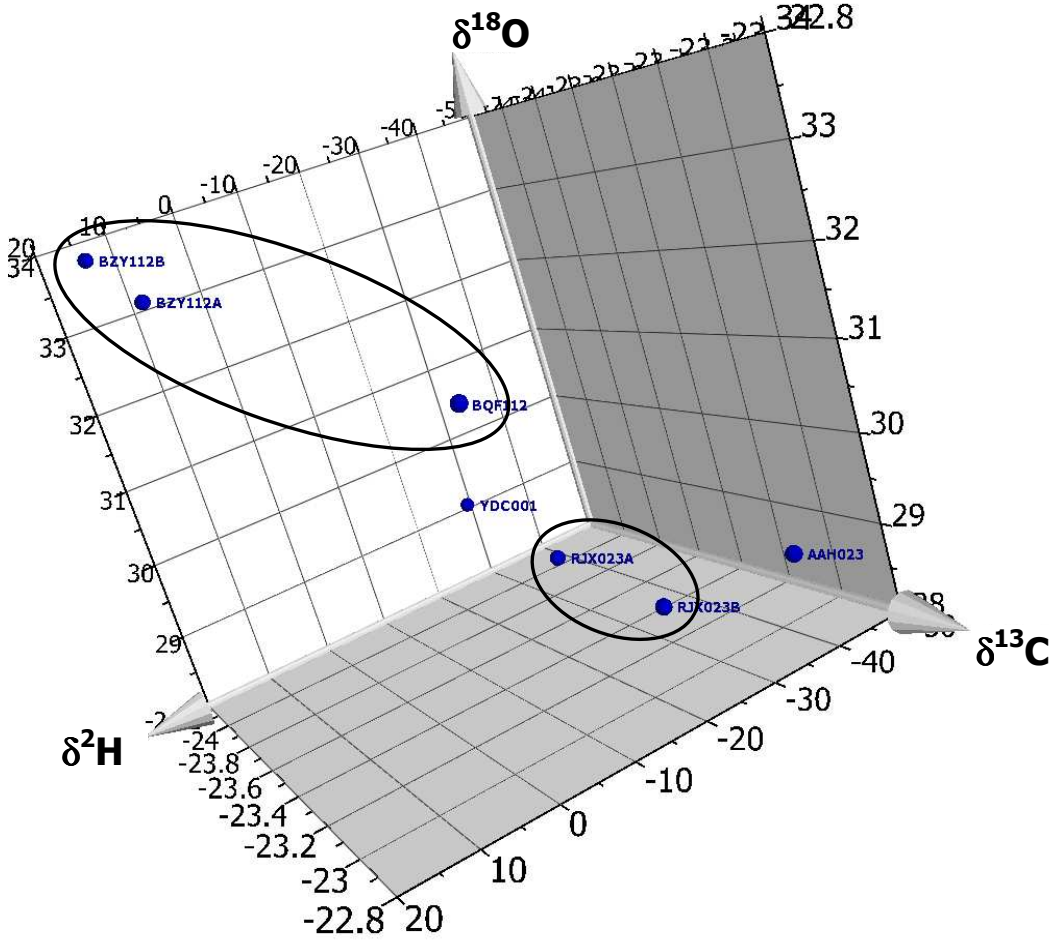

Figure 2

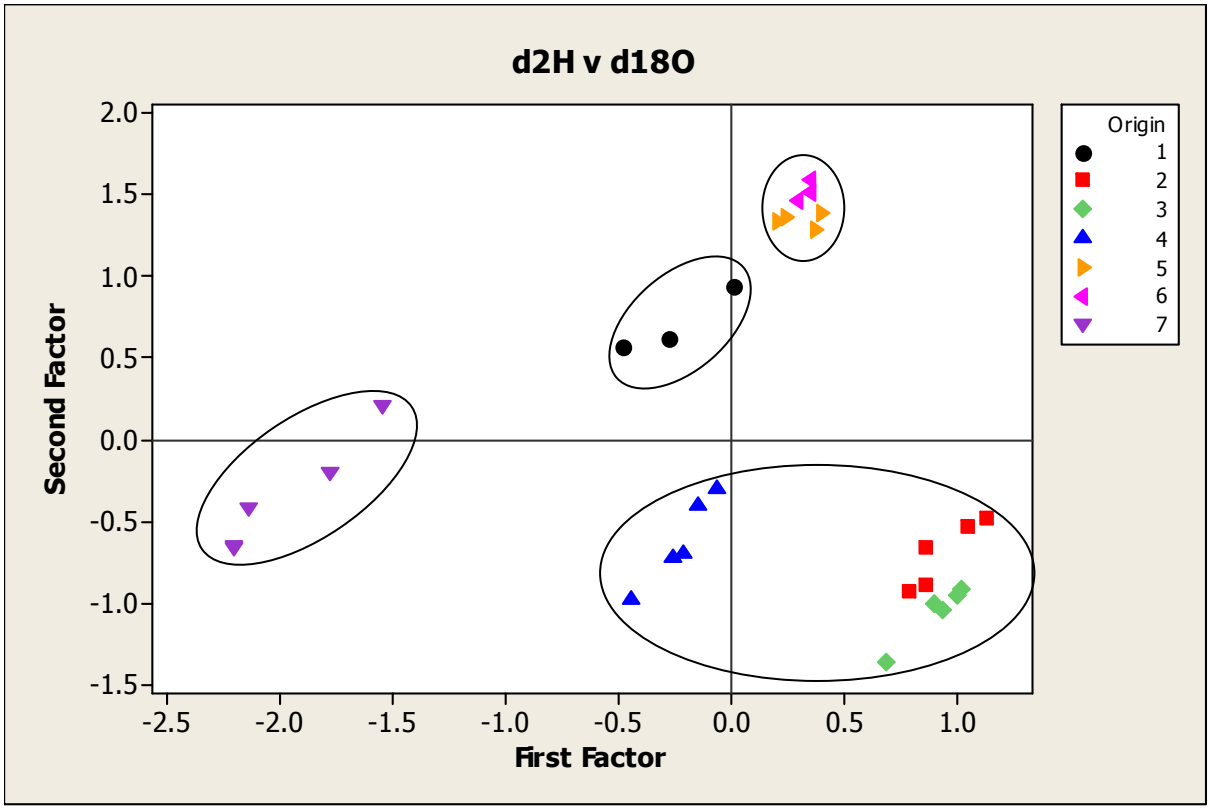

Figure 3