Differentiation of Peats Used in the Preparation of Malt for Scotch Whisky Production Using Fourier Transform Infrared Spectroscopy

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ABSTRACT

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It is not known if peats derived from different areas of Scotland have distinctive chemical constituents that could impact on malt whisky in discernible organoleptic ways. Fourier transform-infrared (FT-IR) spectroscopy was used as a high throughput screening method to investigate discrimination of a large number of peat samples from six different geographical origins around Scotland. The data were analysed statistically (using principal component-discriminant function analysis) and the results showed a difference between peat samples from different geographical origins. Therefore, we have shown that FT-IR spectroscopy provides a quick and simple method for differentiating peat types.

Key words: Fourier transform-infrared spectroscopy, principal component-discriminant function analysis, peat, Scotland.

INTRODUCTION

Peat is best defined as the "light brown to black organic sediment formed under waterlogged conditions from the partial decomposition of mosses and other bryophytes, grasses, shrubs or trees"[23]. Burning of this material during the kilning of malted barley produces a smoke or "peat reek", constituents of which are adsorbed by the malt. After fermentation and distillation, these chemical species impart characteristic flavours to the spirit that can survive maturation and are key quality attributes for certain Scotch whiskies[24]. The peat used by the Scotch whisky industry is currently sourced from various locations across Scotland particularly from the north east of the mainland and the islands of Islay and Orkney.

All peatlands comprise two layers: an acrotelm and a catotelm, the separation of which is defined by the water table[4]. The acrotelm roughly approximates to the upper aerobic layer of between 10–50 cm and the catotelm represents the lower, waterlogged anaerobic layer. In Scotland, peatlands can broadly be divided into climatic bogs and local bogs[8]. Climatic bogs such as blanket bog are typical of western and northern Scotland as they rely on high and consistent rainfall to remain waterlogged and as such are termed ombrotrophic[20]. These bogs are typically characterised by Sphagnum mosses, cotton grass and heathers. Local bogs such as basin or valley bogs do not rely on particular climatic conditions; rather they are formed under the influence of ground water from the surrounding soil and are therefore termed rheotrophic[20]. Local bogs can thus form in any location as long as the inflow of ground water is sufficient to allow plant growth and to retard decay. The chemical composition of peat is influenced by the type of vegetation from which the peat is derived as well as the level of decomposition[2]. These influences on peat composition can be affected by changes in climatic and environmental conditions[2].

It is not known if peats derived from different areas of Scotland have distinctive chemical constituents that could impact on malt whisky in discernible organoleptic ways. In this study we show that peat samples from four geographically distinct areas (Islay, Orkney, north east mainland (St Fergus) and north central mainland (Speyside)) can be distinguished by Fourier-transform infrared (FT-IR) spectroscopy coupled with multivariate statistical analyses.

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MATERIALS AND METHODS

Samples

Approximately 24 transect line samples were taken from between 30 to 50 cm below the surface from a representative area of the six commercial peat extraction sites listed in Table I.

Where peat was being extruded (Gartbreck moss, Castlehill, St Fergus and Tomintoul) a single transect line was used, and where peat was being cut along banks (Glenmachrie moss, Hobbister Hill) transect samples were divided amongst three separate banks to allow an adequate area to be sampled. Depth profiles were taken from the peat banks of Glenmachrie moss and Hobbister Hill, from the surface to the base of the wall. For sample labels the

Table I. Description of peat sampling sites.

Site name	Abbreviation	Location	Grid reference	Deposit type
Castlehill	P	Islay	NR3650	Blanket bog
Gartbreck moss	B	Islay	NR2858	Basin/valley bog
Glenmachrie moss	L	Islay	NR3350	Basin/valley bog
Hobbister hill	H	Orkney	HY3806	Blanket bog
St Fergus	N	Aberdeenshire	NK0553	Basin/valley bog
Tomintoul	T	Speyside	NJ2020	Basin/valley bog

uppercase letter indicates the site, the lower case letter a transect (t) or depth profile (p), and the number indicates the sample number; thus, Tt16 indicates sample 16 of a transect taken at Tomintoul. Commercial samples were also obtained from these sources and are labelled "ind".

Fourier transform infrared spectroscopy

Peat samples were air dried to a moisture content of <20% before initial reduction to a soil-like consistency using a Bosch AXT Rapid 180 garden shredder (Robert Bosch Limited, UK). Samples were then fractionated using a sample divider (Endecotts Limited, UK) and ground to a dust using a SPEX 6700 freezer/mill (Glen Creston Limited, UK). Samples of 0.5 g (±0.0005 g) of each finely ground peat were suspended in 4 mL UHQ water, mixed, and 3 aliquots (10 µL) were placed on a 96 well aluminium plate and dried at 50°C for 30 min.

The plate was loaded onto a motorised microplate module HTS-XT™ attached to an Equinox 55 module (Bruker Optics Ltd., UK). The motorised module of this instrument introduces the plate into the airtight optics of the instrument, in which tubes of desiccant are contained to remove moisture[15]. An MCT (mercury-cadmium-telluride) detector cooled with liquid N_2 was employed for collection of IR spectra in reflectance mode. Spectra were collected in triplicate (giving a total of nine spectra for each sample) over the wavelength range of 4000 to 800 cm^{-1} under the control of a computer programmed with Opus 4, operated under MS windows 2000. Spectra were acquired at a resolution of 4 cm^{-1}, and 64 spectra were co-added and averaged to improve the signal to noise ratio. The collection time for each spectrum was approximately 60 s, the spectra were displayed in terms of absorbance.

In order to account for unavoidable baseline shifts when collecting spectra in reflectance mode, the baseline was corrected using rubber banding (provided by the instrument manufacturers) and the spectra were then scaled linearly so that the minimum absorbance was set to 0 and the maximum to 1.

Multivariate statistical analysis

In this study the aim of the multivariate analysis is to investigate FTIR fingerprint spectra's potential for discriminating between the six different peat geographic locations, and subsequently observing the inter-group pattern of clustering, to establish the extent to which the patterns relate to the geographical location of the sample peat.

In order to build a model which focuses on the differences between peat locations, and ignores extraneous variability in the data, some sort of supervised multivariate discrimination method is needed; where, supervised refers to the use of *a-priori* knowledge about class structure within the modelling process. One such supervised method which has proved robust and reliable with spectroscopic data is discriminant function analysis (DFA; also known as canonical variates analysis (CVA))[17].

DFA is a supervised projection method. *A priori* information about sample grouping is used to produce measures of within-group variance and between-group variance. This information is then used to define discriminant functions which optimally separate the *a priori* groups. These functions can then be used as a co-ordinate system to visualize the DFA scores (i.e. the PC scores projected into DFA space).

DFA cannot be performed on the raw absorbance data set due to mathematical instability. The starting point for DFA is the inverse of the pooled variance-covariance matrix within *a priori* groups. This inverse can only exist when the matrix is non-singular, i.e. its determinant is other than zero, which implies that it is of full rank[6,17]. Generally DFA can only be performed if

$$N_s - N_g - 1 > N_v \qquad (1.0)$$

where, N_s is the number of samples, N_g is the number of *a priori* groups, and N_v is the number of variables. With FTIR data, which typically has ~1600 variables, direct DFA is not possible. One solution is to perform PCA as a pre-processing step. PCA is a well known technique for reducing the dimensionality of multivariate data whilst preserving most of the variance[13].

As the two-stage PCA-DFA is a supervised projection method, some strategy is needed to pick the optimal number of principal components to project into DFA space. The only robust way of estimating the correct number of PCs is by carrying out some sort of cross validation, in this case training set/test set validation. This strategy involves simply performing a linear search. Starting with *m* PC scores (where, *m* is determined by manipulating equation 1.0) a DFA model is built using the training set and validated using the test set. The number of PCs is then reduced by 1 and another DFA model is built and validated. This process is repeated until a set of *m* DFA models have been built. The optimal number of PCs is then determined visually, or statistically, by looking at the DFA scores for each model in turn. The optimal model is the one that provides maximal group separation such that 'test set' members of a given group are correctly classified.

Finally, the Euclidean distance between *a priori* group centres in PC-DFA space was used to construct a similarity measure, with the Gower general similarity coefficient S_G, and these distance measures were then processed by an agglomerative clustering algorithm to construct a dendrogram[19].

In this study all models were built using the Matlab interactive scientific programming environment. PCA was

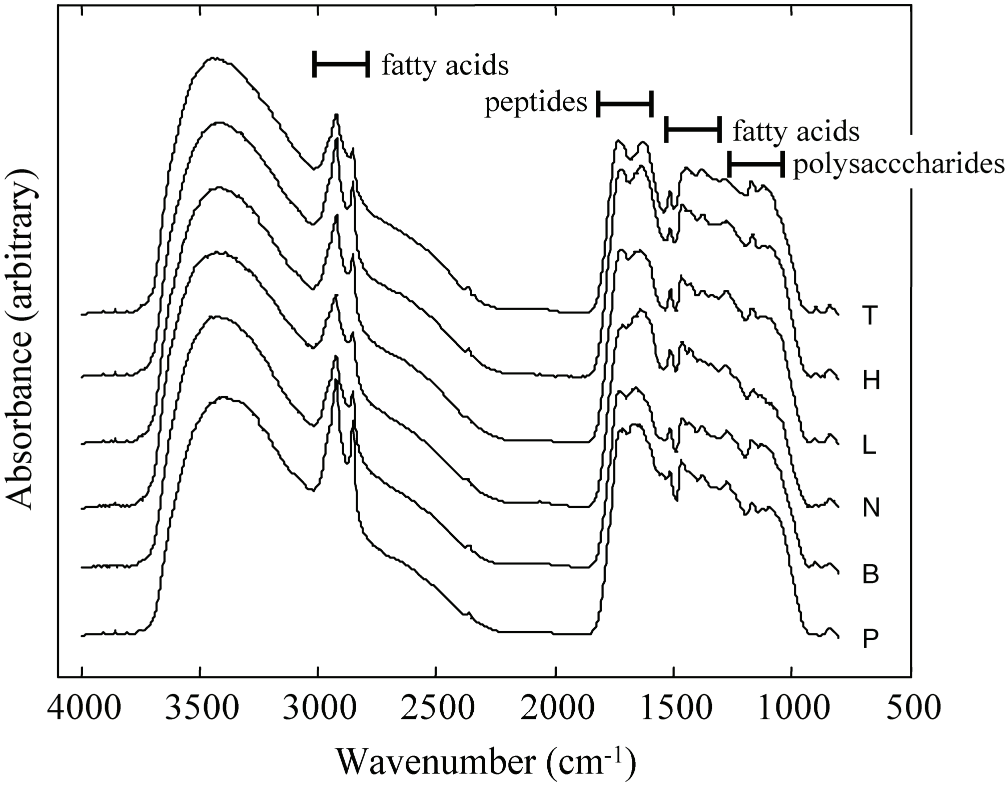

Fig. 1. Typical FTIR spectra from the peat samples from the six different locations, encoded T, H, L, N, B and P (see Table I for details). These spectra are offset so that any differences can be more readily observed.

implemented using the NIPALS algorithm[26]. DFA was implemented as CVA; with the scores normalized to unit within-group variance allowing 95% χ^2 confidence regions to be plotted around each *a priori* class centre[16]. All Matlab scripts are available upon request.

RESULTS AND DISCUSSION

Peats from different sources have previously been differentiated using Curie-point pyrolysis mass spectrometry[1,21]. While this approach provides information on the relative contributions of chemical species, it is destructive and there are serious reproducibility issues[12]. By contrast, FT-IR is a vibrational spectroscopic technique which measures the absorbance of infrared light by functional groups in molecules[14]. FT-IR is rapid, automated, non-destructive and is not biased to any particular group of chemicals and so generates 'holistic' fingerprints of the biological samples under investigation[13,18]. Although FT-IR spectroscopy has been used previously in the characterisation of peat from different depths in a profile[5,11] and distinct vegetation zones in and bordering a Scots pine woodland[3], it has not been used to classify peat from different geographical locations as employed here.

FT-IR spectra

Typical FT-IR spectra produced from the six different peat locations are shown in Fig. 1. Major peaks associated with fatty acids, peptides and carbohydrates can be readily observed as detailed by Schmitt and Flemming[22]; however, all these spectra (and indeed the others collected) show similar broad features with very few visibly discernible qualitative or quantitative differences between them. This illustrates the need to employ multivariate statistical techniques for the analysis of these FT-IR data. Given the complexity of these spectra it is usual that discriminant analysis algorithms are applied so that samples may be classified according to any groups detected in the data.

Discriminant analysis of FT-IR spectra

The spectra were analysed using PC-DFA as described above. Initially the *a priori* knowledge of which spectra were replicates was used as the supervised class structure; thus attempting to remove any analytical variance in the data without forcing the data to conform to some other more constrained classification (in this case peat location) (Fig. 2). While by eye, peat samples from Tomintoul tended to cluster towards the top of the plot, there was no clear separation of samples suggesting that the within 'geographic location' variability is more significant than the between 'geographic location' variability. The other significant finding using this approach was that some samples were atypical and were separated to the left of the bulk of the peats. These atypical samples were all obtained from St Fergus and in some cases the physical appearance (e.g. sample Nt15) suggested a high mineral soil content in place of peat. From the initial PC-DFA results it was apparent that this approach was inadequate for discriminating peat samples by geographic location.

In order to differentiate the peat types according to geographic location we therefore assigned this class structure to the PC-DFA analysis; thus minimising both ana-

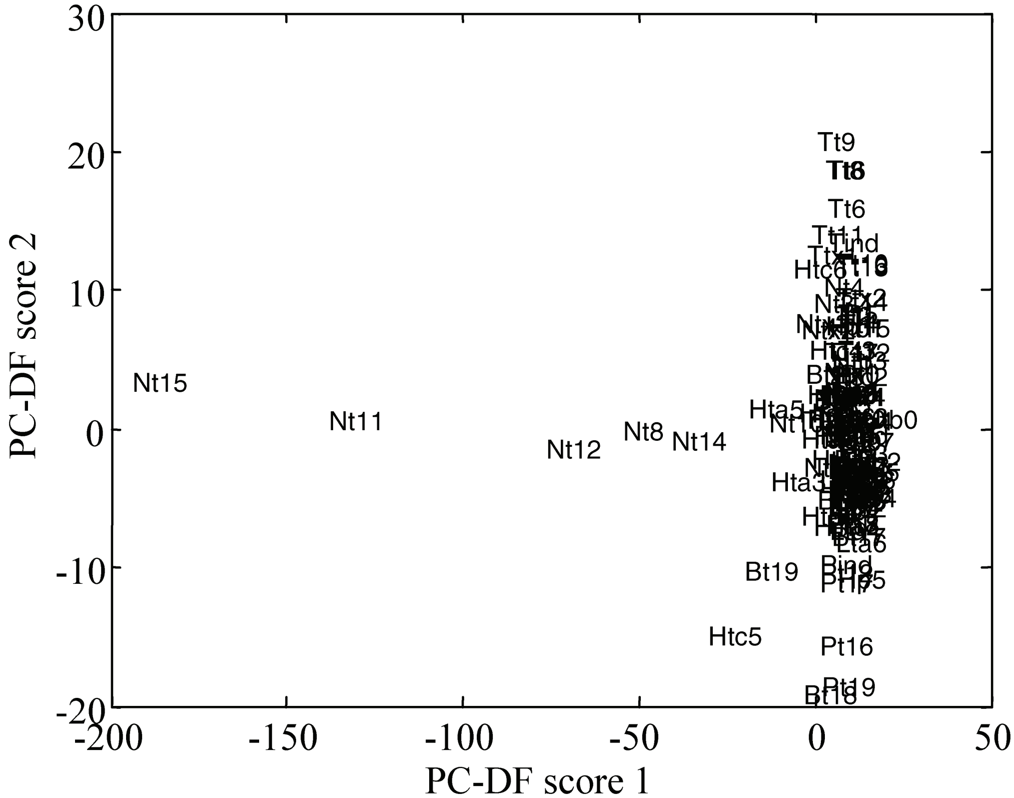

Fig. 2. PC-DFA plot of all peat samples analysed. Samples were assigned individual classes in DFA, and PCs 1–50 used as input to DFA. The means of the nine sample replicates are shown.

lytical variance and within location variance. Taking this approach, peats from two sample sites, St Fergus (N) and Tomintoul (T), could be clearly separated from the others in the first two PC-DF scores (Fig. 3). Whilst the other peats appear to overlap in this plot, when the other PC-DF scores are inspected (data not shown) all peats were significantly different at the 95% confidence level except for peats B and L from Islay (Gartbreck moss (B) and Glenmachrie moss (L)) which were statistically very similar and could not be distinguished.

This result was notable as Tomintoul and St Fergus were the two peat sampling sites on the mainland of Scotland, the two sites being situated in two distinct areas in the north east of Scotland. It was also apparent that Tomintoul and St Fergus separated as much from each other as they did from the rest of the sites. The difference between these two areas may reflect different vegetational inputs to the peat. As Tomintoul and St Fergus are both considered basin bogs, the type of vegetation found in these bogs can vary according to ground water input[8] thus having an impact on the peat chemistry. On inspection, there were found to be differences in the physical appearance of samples taken from the two sites. Of particular interest was the presence of woody remains in several of the St Fergus samples whereas in the Tomintoul samples the levels of recognisable woody material were low. Conversely, some of the samples taken from Tomintoul appeared to contain Sphagnum moss remains. Peats with woody plant origins and peats with moss origin will be chemically distinct. For example, lignin is a polyphenolic structural component characteristic of the tissues of woody plants which in terms of abundance is second only to cellulose in the plant world[7]. Lignin is composed of guaiacyl (monomethoxy), syringyl (dimethoxy) and p-hydroxyphenyl units, the proportions of which are dependent on plant species[7]. Sphagnum moss does not contain true lignin although it is thought to contain an analogous polyphenolic substance lacking guaiacyl and syringyl units[25]. Therefore, peat samples containing a high percentage of Sphagnum moss remains would be expected to be relatively low in mono- and di-methoxy phenyl units.

Samples from the remaining four sites (Hobbister Hill on Orkney (H) and the three Islay sites, Gartbreck (B), Glenmachrie (L) and Castlehill (P)) were from islands and whilst very similar were clustered into three groups; (1) L and B together, (2) P and (3) H. A dendrogram of the group means was constructed from these data along with the peats from the mainland (Fig. 4) which allowed a more quantitative assessment of the relative similarities between these peats to be visualised. Two of these island peat deposits, Gartbreck and Glenmachrie, have been described previously as basin bogs while the other two deposits, Hobbister Hill and Castlehill, are blanket bogs (Table I). The separation of Islay and Orkney samples from the mainland samples collected from the north east of Scotland may reflect the fact that in the relatively acid, low nutrient basin bogs of the west and north of Scotland the predominant plant species are varieties of Sphagnum as well as such plants as sedges, heather and cotton grass which are species also common to blanket bogs[8]. Moreover, they are all formed on islands under the similar climatic conditions of a cold-temperate oceanic climate. It

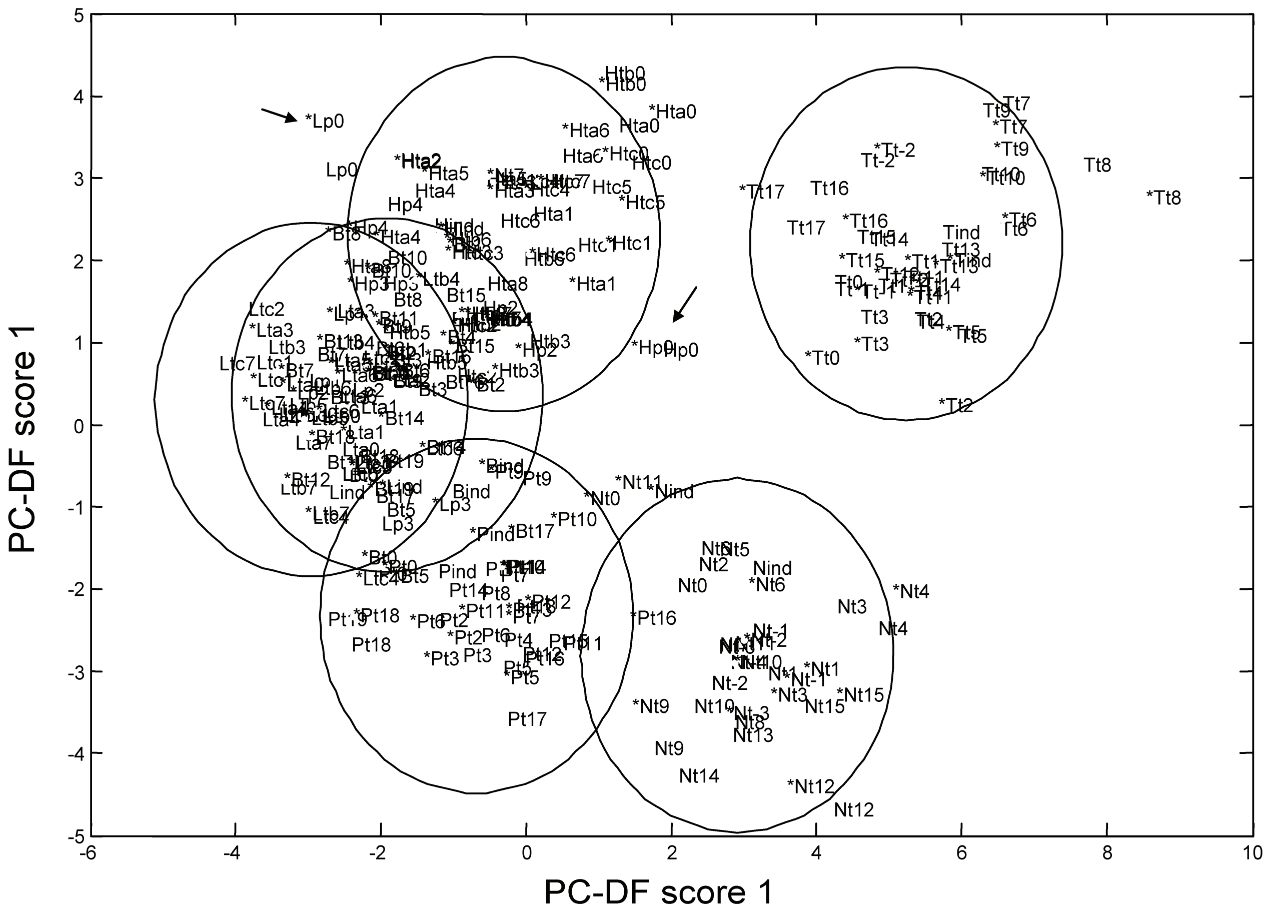

Fig. 3. PC-DFA plot of all the peat samples encoded with knowledge of the six geographical locations from which the peat was collected. PCs 1–80 used as input to DFA. Training sets are denoted by symbols (e.g. Nt4) and test set by symbols and asterisks (e.g. *Nt4). Circles represent 95% χ^2 confidence limits. Arrows represent outlier samples that were from upper horizon of the peat depth profile.

was however, hard to establish any obvious general differences in physical appearance between island and mainland peat that could confirm this speculation.

As can be seen in the dendrogram (Fig. 4), samples from the three Islay sites clustered together and were separated from the Hobbister Hill samples from Orkney. This may reflect a small difference in the composition of peat from Orkney and Islay. It has been noted previously that heather tends to be relatively more abundant in peat deposits in the North of Scotland compared with the south west[9]. An increased prominence of a particular plant species such as this may have an effect on the chemical composition of the peat.

Finally, the peats from Islay clustered into 2 groups which were related to either coming from a blanket bog (Castlehill) or from a basin/valley bog (Gartbreck and Glenmachrie). Indeed, samples from Gartbreck and Glenmachrie were indistinguishable from each other in the dendrogram (Fig. 4) and PC-DFA ordination plot (Fig. 3).

To assess the effect of cutting depths on peat composition, samples were taken from different depths at Glenmachrie and Hobbister Hill – the two sites where peat is extracted along banks from all depths simultaneously. In both cases the samples taken from the upper horizon of the depth profile, Lp0 and Hp0 respectively were recovered outside the 95% confidence intervals (indicated by arrows) and so are distinct on the PC-DFA plot from all other samples taken from these sites (Fig. 3). This probably reflects the fact that both Lp0 and Hp0 contain material that has only relatively recently entered the acrotelm and as such has not been exposed to the same level of decomposition as the other samples. The underlying samples, on the other hand, are likely to be more similar to each other due to the slowing of the decomposition rate as peat passes into the anaerobic catotelm[10]. The fact that all of the samples – other than the uppermost which were taken from a depth not used for peat burning – in each depth profile were typical of the site indicated that depth has little impact on the chemical composition of the peat.

Finally, samples were taken from stocks of previously cut peat at each site to see how their composition related to that of the transect line samples. These samples had been used in the PC-DFA shown in Fig. 3, and that they all co-localised with the transect samples from their respective sites showed that industrially cut peat shared similar chemical characteristics with the transect samples and that peat samples could be identified by FT-IR to a principal location.

In conclusion, FT-IR spectroscopy coupled with cluster analysis allowed the classification of peats according to their geographical origin. The implication is that peats

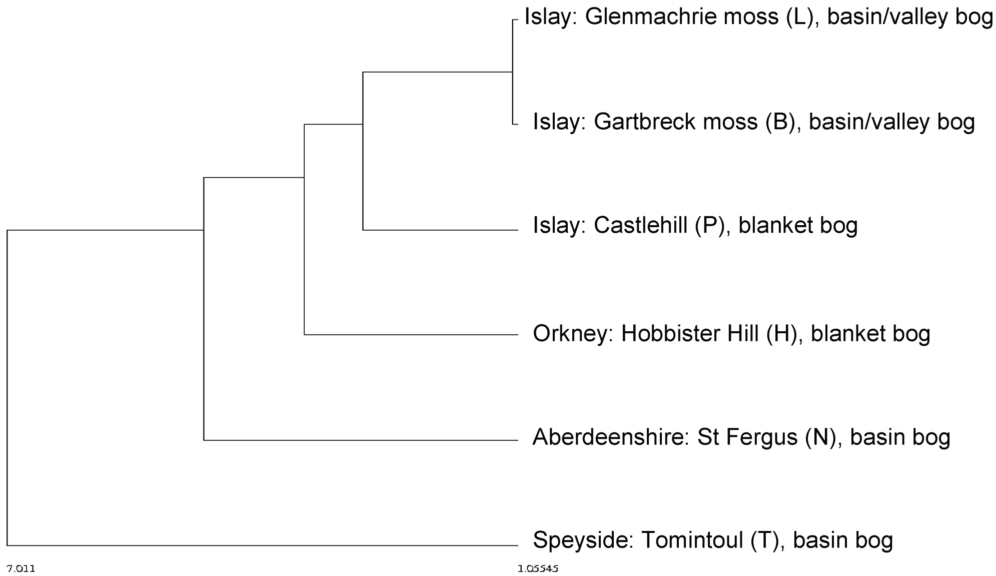

Fig. 4. Dendrogram constructed from the PC-DFA group means showing the relationship between the six peat samples.

from different origins may contribute different chemical characteristics to peated malt and, therefore, to peated Scotch whisky. However, although multivariate statistical analysis was able to discriminate between apparently identical FTIR spectra, the significance of these results with respect to peated malt remains uncertain until similar discrimination is found between the products of pyrolysis from the same samples of peat.

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