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Title Page (with authors and addresses)

Elemental and Molecular Profiling of Licit, Illicit, and Niche Tobacco

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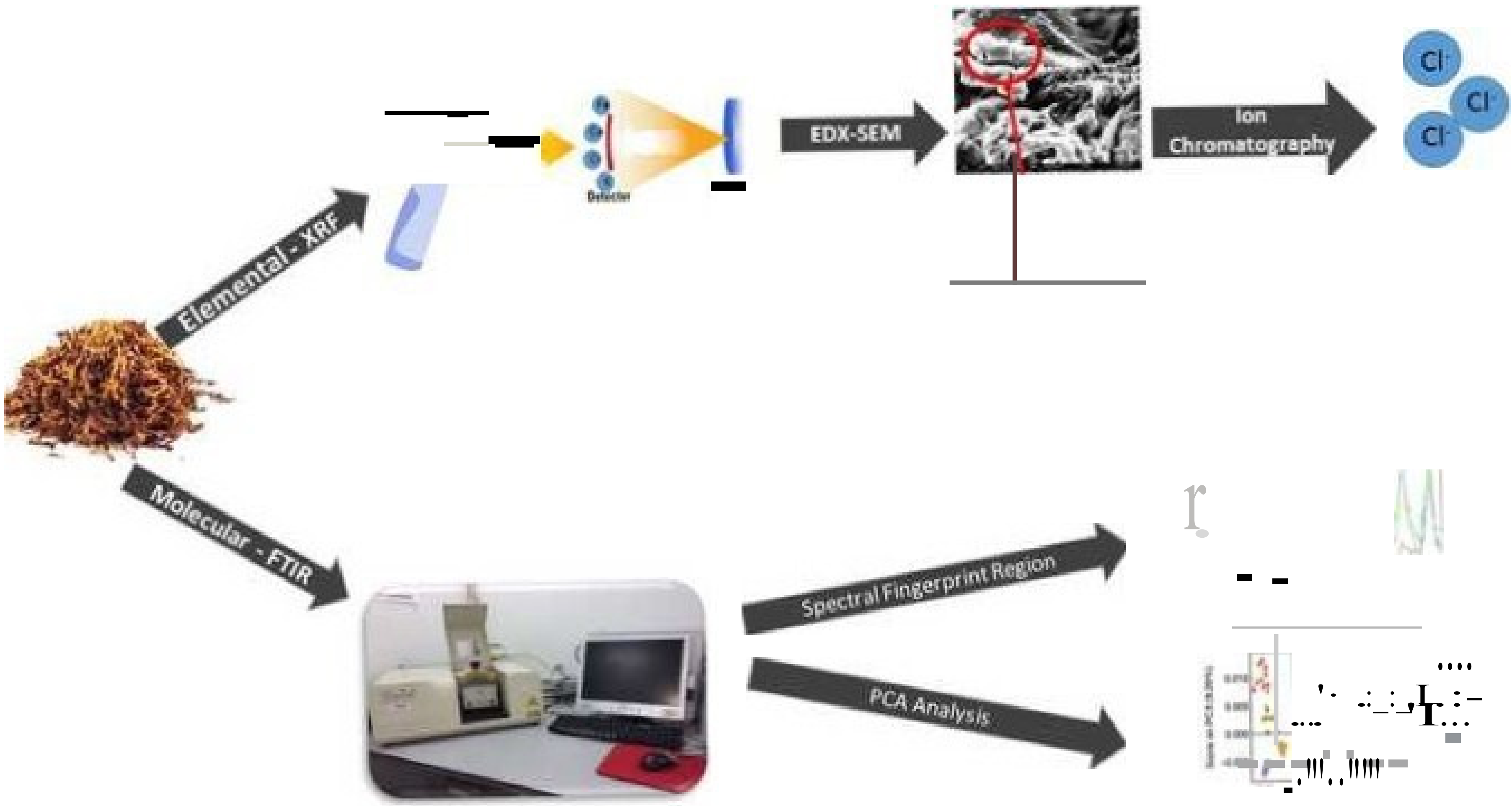
Graphlca Abstract (for review)



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Highlights

- The identification of Ca, Cl, K and Fe as key elemental indicators of tobacco provenance based on analysis using handheld XRF using samples of licit, illicit and niche tobacco donated by Lancashire Trading Standards.
- The identification of a highly discriminative FTIR tobacco spectral fingerprint region, based on absorptions attributed to carbonyl stretching at 1050-1150 cm^{-1}, alkane bending at 1350-1480 cm^{-1} and amide I stretching at 1600-1700 cm^{-1}.
- The imaging and quantification of Cl^- crystalline structures found within Swedish Snüs tobacco.

Elemental and Molecular Profiling of Licit, Illicit, and Niche Tobacco

Abstract

The recognition of differences between regulated large-scale mass manufactured products and the uncontrolled cultivation of tobaccos for illicit purposes plays a significant role within identification of provenance. This research highlights X-ray fluorescence and Fourier transform infrared spectroscopy as useful analytical techniques for the rapid identification of tobacco samples of unknown provenance. Identification of key discriminative features within each technique allowed for the development of typical characteristic profiles for each type of tobacco. Analysis using X-ray fluorescence highlights chlorine, potassium, calcium and iron as key elemental indicators of tobacco provenance. Significant levels of chlorine seen within Snüs samples prompted attempts to visualise chlorine containing regions and structures within the sample. Scanning electron microscopy images showed crystalline structures visible within the Snüs tobacco, structures which Energy dispersive X-ray Spectroscopy qualitatively confirmed to contain chlorine. Chloride levels within Snüs samples were quantified using ion chromatography with levels found to range between 0.87 mg mL^{-1} and 1.28 mg. Additionally, FTIR indicated that absorbances attributed to carbonyl stretching at 1050-1150 cm^{-1}, alkane bending at 1350-1480 cm^{-1} and amide I stretching at 1600-1700 cm^{-1} highlighting a spectral fingerprint region that allowed for the clear differentiation between different types of tobaccos using PCA analysis, but was limited by differentiation between provenance of cigarettes and hand rolled tobacco. X-ray Fluorescence and Fourier transform infrared spectroscopy yielded different information with regards tobacco discrimination and provenance, however both methods overall analysis time and cost reduced indicating usefulness as potential handheld analytical techniques in the field.

Keywords: Tobacco, Illicit Tobacco, FTIR, XRF, Elemental Analysis, Spectroscopy

1 Introduction

1.1 Licit, Illicit, and Niche Tobacco

Illicit tobacco is typically sold in the form of cigarettes or hand rolled tobacco, which is grouped by U.K. trading standards and Her Majesty's Revenue and Customs (HMRC) into two main groups: counterfeit products, or 'Cheap Whites' [1, 2]. Counterfeit tobacco products mimic licit brand packaging in an attempt to masquerade as licit products and contain low- grade unregulated tobacco, which is sold to unsuspecting consumers. In comparison, 'Cheap Whites' are cigarettes that utilize poor filters and low grade tobacco marketed under illicit brand names purely targeted for sale to the U.K. illicit market [1].

Niche tobacco products vary drastically in content depending on the desired method of ingestion, where the product can be consumed without full or any pyrolysis [3]. Niche tobacco is a source of licit tobacco from another country consistently prohibited from sale on the U.K. market. These products do not typically meet standards set out in U.K. or European legislation due to limited knowledge of adverse health effects and contents information [3]. Niche tobacco has over time increased in popularity all over the world, predominantly due to the nature of socialization associated with its use [4, 5].

1.2 Elemental Analysis of Tobacco

Many of the chemical substances that are associated with the tobacco plant are attributed to atmospheric depositions or the application of phosphate fertilizers and sewage sludge [6, 7]. The International Agency for Research on Cancer has highlighted tobacco as a major source of known cancer causing heavy metals with such as cadmium and lead. These heavy metals are found within the body adipose tissues after long term accumulation and are linked to life threatening non-cancerous toxicity of the cardiovascular and renal systems [8]. Tobacco plants are highly susceptible to the accumulation of bioavailable elements such as cadmium (Cd), lead (Pb), and zinc (Zn) through preferential uptake mechanisms whereby the presence of one mobile element within the soil will stimulate the uptake of others [7, 9]. Levels of these bioactive elements decrease in the following order throughout the tobacco plant: roots > leaves > fruits > seeds [10]. The interaction of Cd^{2+} and Pb^{2+} with sulphur-hydrogen bonded groups inactivating enzymes to disturb the metabolic process within the cell [7, 10]. Unlike organic materials found in soil, inorganic impurities are not usually removed from a source by chemical or microbial degradation [11, 12]. Elemental fingerprints are typically detected using inductively coupled plasma mass spectrometry (ICP-MS), however, this technique is not practical for rapid on scene diagnostics that are required by U.K. Trading Standards and HMRC due to time constraints and the need for aggressive digestion methods that destroy evidence vital for criminal convictions [8, 13]. X-Ray fluorescence (XRF) has previously been scaled down and applied for safe handheld use as an economical and sensitive technique to provide a bulk elemental profiles of plant foliage in under 15 seconds, easily translatable to tobacco analysis [14-16].

1.3 Spectral analysis and identification of plant material

In some cases, especially to the untrained eye, it is extremely difficult to distinguish visually between licit and counterfeit tobaccos. However, examinations conducted by experienced officers and knowledge of illicit packaging trends at seizure allows for a subjective decision to be made with regards prosecution charges against criminals [17]. A rapid, simple, spectroscopic method of tobacco analysis has the potential to establish a platform for highly discriminative identification of provenance. Due to the complex chemical mixtures found within plant foliage, it is not currently possible to definitively isolate a single absorption band and attribute it to a specific plant constituent, such as chlorophyll, when comparing different types of tobacco [18]. New advances in Fourier transform infrared spectroscopy (FTIR) that have led to improved detection limits and resolution allows for the determination of minor changes in the alkaloid fractions of tobacco, a method which has been adopted by the tobacco quality control industry for the identification of tobacco disease within plants during the incubation period [19].

This research highlights 'user-friendly' rapid methods of tobacco provenance determination in an effort to reduce current costly protocols and potential conflicts of interest with regards current costly outsourced laboratory tobacco analysis using ICP-MS, gas chromatography – mass spectrometry and isotope ratio – mass spectrometry (IR-MS). This research also offers insights into techniques that do not require the destruction of evidential samples by extraction and digestion. By focusing on differentiation between whole spectra, rather than the specific absorbance's of target alkaloids, this research utilizes a simple analytical technique in tandem with multivariate data analysis to highlight tell-tale fingerprint regions that identify tobacco sample provenance.

2 Materials and Methods

2.1 Samples

Samples of varying provenance (79 in total) were donated by Lancashire Trading Standards or purchased from a variety of licit retailers across the U.K., France, Spain, Belgium, Germany and Sweden. All samples donated by Lancashire Trading Standards were not utilised as evidence in active cases and were the maximum number of samples made available to the researchers throughout the duration of this study. The samples included illicit, duty free, and niche tobacco and were organised into categories based on the brand and seizure type. A list of definitions for the different types of tobacco analysed within are listed in table 1 (for detailed sample information and quantities please see supplementary information). Prior to analysis each sample was separated for triplicate analysis, dried in an oven at 70°C for 30 minutes, and ground into a fine powder using a pestle and mortar. Any unused tobacco samples were incinerated upon completion of this study at the request of Lancashire Trading Standards.

Table 1. Definition, descriptions, and quantity of different types of tobacco included within this study, all definitions are adapted from the NTPD website [3], http://www.ntpd.org.uk accessed on 08/11/2015 at 23.07.

Tobacco	***Definition and Description***	***Number of Tobacco Samples within study***
Cigarettes	Cigarettes are the most common tobacco product available on the market, made up of flakes of tobacco leaf that have been rolled into a cylindrical shape using a filter and thin filter paper	45
Miniature Cigars	Miniature cigars are roughly the same size as a cigarette, made using large tobacco flakes rolled in whole tobacco leaf to allowing for a slower rate of pyrolysis	2
Hand Rolled	Hand rolled tobacco is usually a blend of several types of tobacco, with thin wiry strands that are rolled using filter paper into a cigarette form or alternatively smoked in a pipe	16
Khaini	Khaini has a predominantly male market within India and Pakistan. Consumed socially, the user combines the Khaini by pressing it into the form of a ball, then places it in the oral cavity where it is then held and sucked occasionally for 10-15 minutes. Khaini contains fragments of leaf material, tobacco, slaked limed paste and areca nut	2
Gutkha	India and Pakistan are the main retailers and consumers of Gutkha products, with a target market of young men and boys. Gutkha is sucked, spat or chewed and typically contains betel nut, catechu, tobacco, lime, saffron and additional flavouring agents specific to the brand	4
Snuff	Fire cured tobacco, more commonly known as snuff, is found in a dry	2

	powdered form with less than 10% moisture content which is then either sniffed or held in the oral cavity. Snuff is a product typically found within the U.K, USA, India and Sweden	
Snüs	Predominantly produced in Sweden on a large scale, Snüs is found in either loose or pouched form with a typical portion being between 0.5g-1.0g. The pouch or loose tobacco is held in between the wet membrane of the gum and cheek to allow for rapid absorption of constituents into the blood stream. The tobacco is finely ground, dried and mixed with aromatic substances, salts, humidifying agents such as Sodium Carbonate, additional nicotine and water	5
Shisha	Shisha or Water pipe tobacco, the composition of which varies and is typically found to have thick almost bark like fragments of tobacco leaf mixed with artificial flavourings. Additional nicotine and aromatic compounds are present resulting in a sticky oily residue that is used to give a specific desired fragrance to the tobacco. Shisha tobacco is marketed based on flavour/fragrance and is typically produced in North African countries, Eastern Europe and Southern Asia	3

2.2 Materials and Methods

2.2.1 X-ray Fluorescence

X-ray fluorescence (XRF) sample vessels were lined with X-ray grade film (Mylar, U.K.) before addition of 250 mg dried and ground tobacco sample; the film was replaced between samples and vessels were cleaned with ethanol (99% Sigma Aldrich) prior to analysis. All tobacco samples were analysed in triplicate with a Bruker Handheld XRF Tracer IV-SD (Bruker, Germany) at 40 keV, 15 µA over 15 seconds with peak assignment based on alignment with K-Line energy transitions.

2.2.2 Energy-dispersive X-ray Spectroscopy – Scanning electron microscopy

Surface features of licit whole leaf tobacco and Swedish Snüs ground niche tobacco elemental analysis (including imaging) was performed with a FEI QUANTA 200 EDX-SEM (FEI, Netherlands). Snüs samples were utilised for analysis due to unexpected chlorine levels identified within Snüs samples only during XRF analysis. Whole leaf licit cigarettes samples were utilised to aid a comparison against standardised licit tobacco. Samples were dried and sputtered with a 5 µm layer of gold using an EMITECH K550X (EMITECH, U.K.) gold sputter coater.

2.2.3 Ion Chromatography

Only samples of Swedish Snüs tobacco were used for Chloride (Cl^-) analysis with ion chromatography with 350 mg of each sample (loose) measured into an individual beaker with 75 mL de-ionised water on a hotplate at 100°C for 10 minutes with a magnetic stirrer bar; 1 mL of the extracted solution was diluted with 9 mL of deionised water. The standard calibration curve was prepared with a 500 mg mL^{-1} stock solution using Multielement Ion Chromatography Anion Standard solution (Sigma Aldrich, U.K.) for quantitative Cl^- analysis with a Dionex ICS-2000 RFIC ion exchange chromatograph (Thermo Scientific, U.K.). Samples were introduced manually and the instrument parameters were as follows: mobile phase potassium hydroxide (KOH) (Sigma Aldrich, U.K.) & deionised water, data collection rate of 5.0 Hz, cell temperature of 35°C, 4 X 250mm Dionex Ion Pac AS17 column (Thermo Scientific, U.K.), column temperature of 30°C, ASRS 4 mm suppressor, suppressor current of 75 mA, pressure lower limit 200-3000 psi, with a flow rate of 1 mL min^{-1}. No guard column was utilised for this analysis.

2.2.4 Fourier transform infrared spectroscopy

The instrument used for FTIR analysis of tobacco was a Specac Golden Gate Attenuated Total Reflection (ATR) stage (Specac, U.K.) and a Jasco FT/IR 410 (JASCO, U.K.) recording absorbances between 650-4000 cm^{-1} with a resolution of 4 cm^{-1}, 1 spectrum represents 64 added scans. Each tobacco sample was placed directly onto the ATR stage and crushed beneath the clamp; the ATR stage was cleaned using ethanol (99% Sigma Aldrich) prior to each analysis to ensure the crystal was free from contamination.

3 Results and Discussion

3.1 Elemental Profiling

Calibrated quantitative analysis of XRF spectra is demanding for even the most competent of users thus spectra was qualitatively analysed to reduce data processing times as well as being easier to translate to potential end-users. Elements typically associated with soil attributions such as K, Ca, Mn, Fe, Ni, Cu, Br and Sr were observed within all spectra and peaks were assigned within each spectrum based on the K-line energy transitions only. Compton scatter, sum peaks and escape peaks encountered were identified and discounted from analysis as per standard practice. Averaged count rates of Cl, K, Ca and Fe were highlighted as the key elemental indicators of different tobacco provenance (see figure 1). Illicit 'Cheap White' and counterfeit cigarettes were distinguished from their licit counterparts by an increase of 1000 counts associated with Ca and an averaged increase between 200-300 counts related to Fe. Illicit hand rolled tobacco was differentiated by an increase in levels of Cl (200 counts), K (2000 counts), Ca (2700 counts) and Fe (500 counts). Elemental levels of niche tobacco varied based on the constituent ingredients and preservatives, for example niche tobaccos known to contain areca nut, betel nut, and slaked lime such as Khiani and Gutkha exhibit drastic increases in K (Khiani, 17000 counts) and Ca (Gutkha, 12000 counts), respectively. Levels of Cl exhibited within the Swedish Snüs tobacco was of interest due to the assumption that pre-treatment throughout manufacture was minimal as it appeared tobacco was only ground throughout production. However, the samples of Snüs exhibited averaged count rates of Cl that were double that of a licit cigarette (4000 counts).

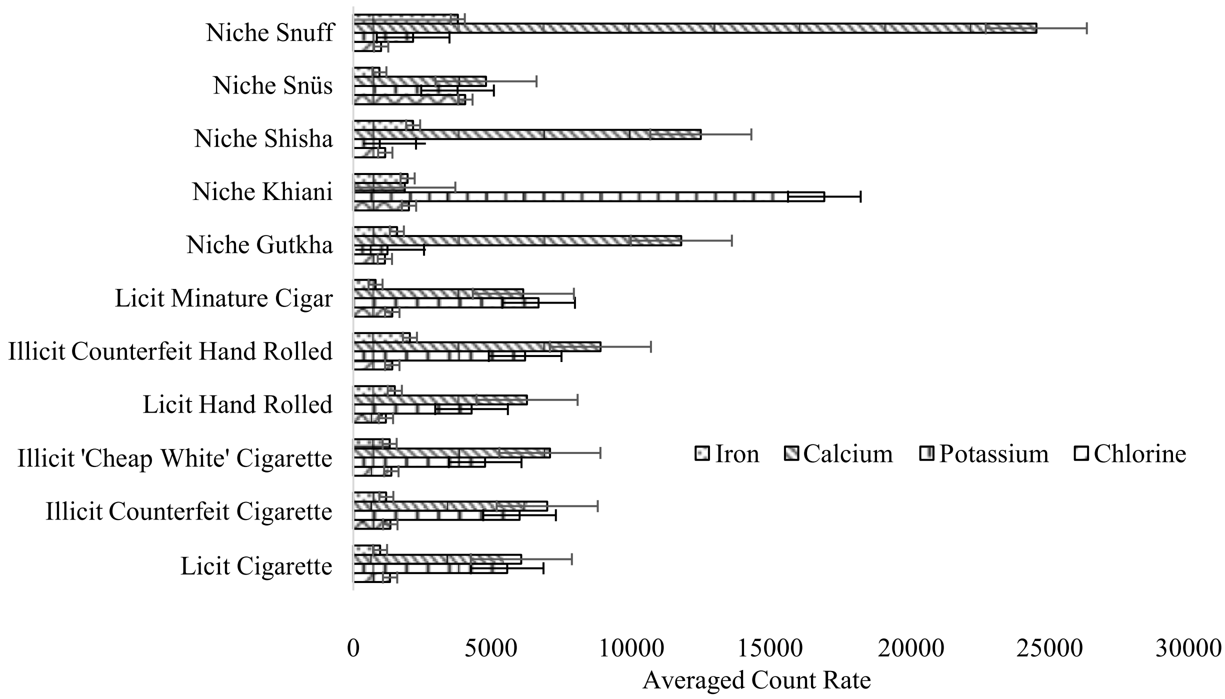
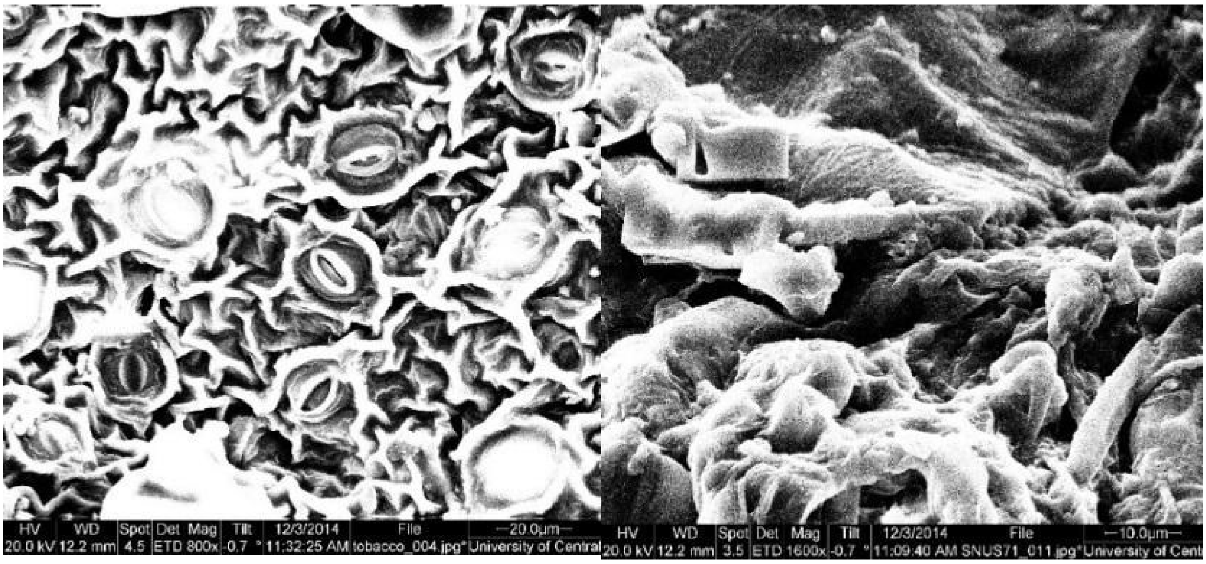


Figure 1. Average count rates, including averaged actual error, for each different type of tobacco provenance based on the four elements that showed the greatest visual variance during XRF analysis.

Levels of Cl identified amongst the Snüs tobacco samples of the XRF study conducted within this research, prompted attempts to visualise regions and structures within the tobacco that differentiate from licit whole leaf samples. (i) Whole leaf tobacco and (ii) Snüs tobacco using SEM at 800× magnification is illustrated in figure 2. Snüs tobacco is the least treated of the niche tobaccos and still visibly contains tobacco leaf; however, it can clearly be differentiated from whole leaf cigarette tobacco. The stomata are clearly visible and undisturbed within the licit leaf tobacco in comparison to the treated Snüs sample. Crystalline structures were present within the Snüs tobacco at 1600× magnification confirming the high levels of Cl using EDX, shown in figure 3. Building on the results from prior X-ray fluorescence and EDX-SEM analysis, levels of Cl within each Snüs tobacco sample were quantified using ion chromatography in order to determine if these levels have the potential to cause adverse health effects when compared to traditional tobacco. Chloride ions eluted from the column at 2.4 mins and levels found within the Swedish Snüs tobaccos varies between 0.87 mg mL^{-1}

and 1.28 mg mL^{-1}. Levels of Cl^- were not observed in excess within any of the other treated or untreated tobaccos and as such, it is assumed that the chloride is added in the form of a desiccant in order to preserve and retain the moisture within the tobacco in the form of calcium chloride or potassium chloride. Palladium (Pd) levels observed within the EDX spectra Snüs samples is to Palladium (II) containing residual pesticides/ fungicides introduced throughout the manufacturing process. Palladium (II) pesticides, well known to be incredibly effective low dose anti-fungal and anti-bacterial agents, have no effect to humans or plants at these concentrations and are not cause for concern within this study [20].



(i) (ii)

Figure 2. (i) SEM image of whole leaf licit cigarette tobacoo with stomata visible (ii) SEM image of treated niche Swedish Snüs tobacco with visible crystalline structures used for later elemental analysis using EDX.

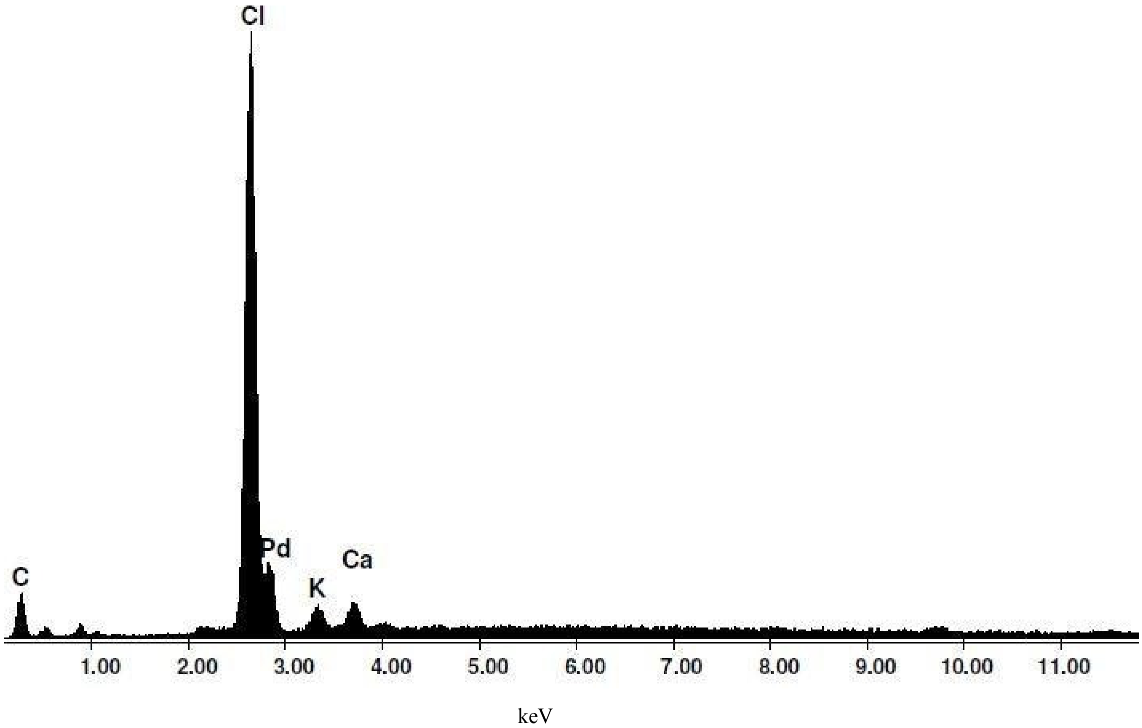


Figure 3. A qualitative EDX elemental profile of crystalline structures found within Swedish Snüs tobacco. Note: the intense Cl peak at 2.65 is thought to be attributed to potassium chloride or calcium chloride salts. Levels of Pd present are thought to be attributed to residues of routinely utilised Pd (II) containing pesticides.

3.2 Spectral Profiling

Pre-processing and multivariate data analysis was carried out on the raw data using MATLAB version 7.11.0 (R2010b) (The MathWorks Inc., USA) using in-house written software and the Spectroscopy Toolbox (Birmingham City University, University of Strathclyde). Groups of niche, cigarette and hand rolled tobacco data were compared to licit cigarettes as these are more likely to have higher levels of consistency between samples due to being from a controlled cultivation plant source. Vector normalization was applied to all the grouped raw data using the Spectroscopy Toolbox [21]. Variable ranking was applied using the Spectroscopy Toolbox allowing for the retention of the top 30% highest discriminatory wavenumbers. From this 30%, the top 30 wavenumbers listed were used to determine functional groups with the most significant differences between the spectra, identifying a spectral fingerprint for each type of tobacco [21]. Principle component analysis

(PCA) using a covariance matrix was conducted after the following data pre-treatment in Mathematica 10 (Wolfram Research, USA); FTIR data was rescaled between 0-1 and the second derivative of the Savitzky-Golay 2nd order polynomial curve 3 point window [22].

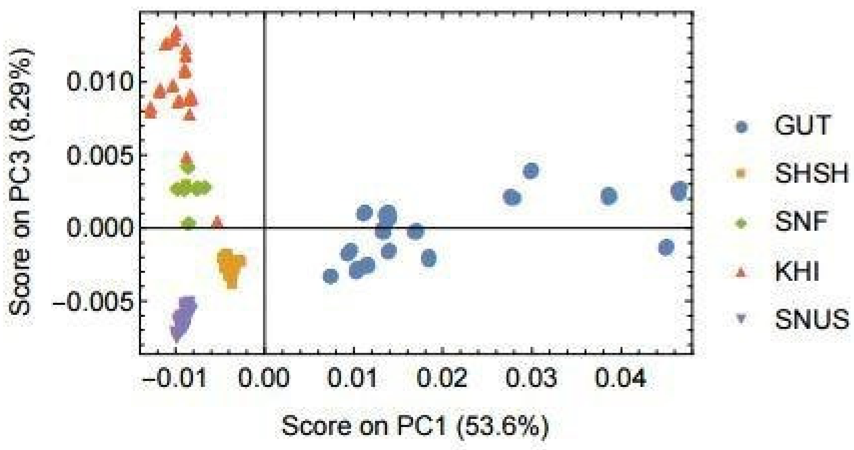
3.2.1 Spectral fingerprinting

The spectra were similar in the respect that absorbances were consistent at the same wavenumbers throughout the different data groups, indicating these highlighted regions were typical of tobacco plant foliage, even in the treated niche samples. Using data analysis software, highest discriminative wavenumbers were ranked to aid comparative analysis. The most significant differences between absorbances from the different types of tobacco samples were recorded to be within the regions of 1050-1150 cm^{-1}, 1350-1480 cm^{-1} and 1600-1700cm^{-1} and related to C-O, -C-H, and C=O. This method was however restricted upon PCA analysis due to the sheer variation between licit and illicit cigarette tobacco blends, producing uneven dispersion even after pre-treatment of the dataset. Discrimination between niche tobaccos is clear as shown within figure 4 below and highlights FTIR as a rapid method of distinguishing these samples from cigarette or hand rolled tobacco samples.

Table 2. Specific absorbances identified after data pre-processing and multivariate data analysis using variable ranking, highlighting the tobacco spectral fingerprint region.

Functional Group	***Absorptions***	***Vibrational Mode***
C-O	1050-1150 cm^{-1}	Carbonyl Stretch
-C-H	1350-1480 cm^{-1}	Alkane Bending
C=O	1600-1700 cm^{-1}	Amide I Stretching
C-H	2850-3000 cm^{-1}	Alkane Stretch
O-H	3200-3600 cm^{-1}	Alcohol Stretch

i)



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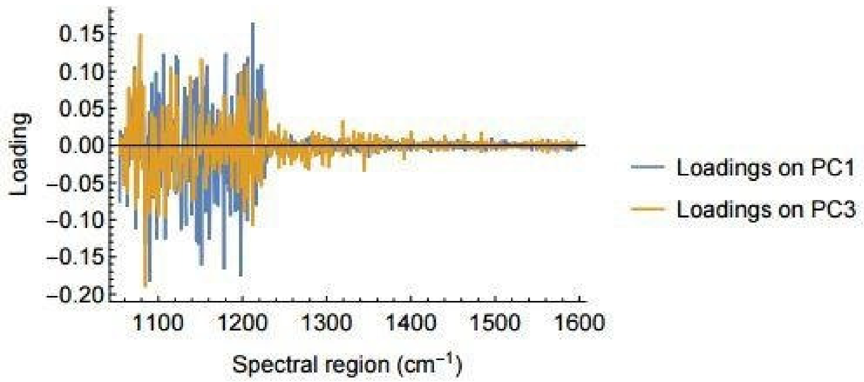

Figure 4. (i) Score and (ii) loading plots of niche tobacco samples, Gutkha, Shisha, Snuff, Snüs and Khaini using PC1/ PC3 loadings of data identified by variable ranking as the tobacco spectral fingerprint region. Plots of PC1 (53% variance) vs PC3 (8%) were used as they show an increased discrimination between the different niche tobacco products when compared to that of PC1 vs PC2 (15% variance).

Both FTIR and XRF have the opportunity to be adapted for handheld use by the non-scientific user for on scene tobacco provenance identification. Fourier transform infrared spectroscopy highlighted a clear discrimination between niche tobaccos upon PCA analysis, where XRF is limited to the discrimination between low averaged count rates of these complex samples that contain a variety of ingredients supplementary to tobacco within the sample. Comparably, XRF allowed for the differentiation between cigarette and hand rolled tobacco blends by rapid qualitative elemental analysis of averaged count rates of K, Cl, Ca and Fe, where FTIR failed to distinguish provenance fully between the variation of blends and samples. The trading of illicit tobacco is not perceived to be an immediate serious issue in comparison to international drugs trafficking or money laundering, but in reality due to the significantly delayed onset of adverse health effects it is more of a threat to the lives of millions than the two put together [2]. Low weighted penalties typically served by the courts and easy payment of fines, criminals are able to make allowances for a predetermined financial risk within the profit margin of each shipment of tobacco. The market is therefore essentially allowed to continue trading without any serious implications, thus generating an easily lucrative trade, which is attractive to terrorist funding operations and organised crime gangs [23]. Currently, confirmatory analysis of tobacco seizures by trading standards and HMRC is outsourced to private companies that are aligned with the tobacco industry for analysis slowing processing and prosecution times, as well as highlighting conflicts of interest [1]. It is shown here that the utilization of new advances in handheld spectral technology, including XRF coupled with FTIR analysis are compatible with advanced multi variate data analysis. This has great potential to develop a spectral library that has high sensitivity and is able to discriminate between minute differences in absorbance between the types of tobacco and to greatly assist the efforts of trading standards and HMRC [24, 25].

4 Conclusions

Although detection limits currently are no match for established analytical laboratory techniques, such as ICP-MS and IR-MS, this research establishes a platform for the spectroscopic determination of differences between different types of tobacco. Utilizing FTIR and XRF as rapid analytical methods of provenance identification provides the potential to develop a spectral library of tobaccos to aid rapid identification of unknown samples. The observation of palladium within EDX spectra sees the detection of residual palladium (II) containing pesticides, which are considered high-grade and utilised by the Swedish tobacco industry. This observation alone highlights the potential for a new method of rapid tobacco provenance identification based upon pesticide and fungicide residues utilising rapid handheld elemental and molecular profiling as described by the methods within this study.

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Figure

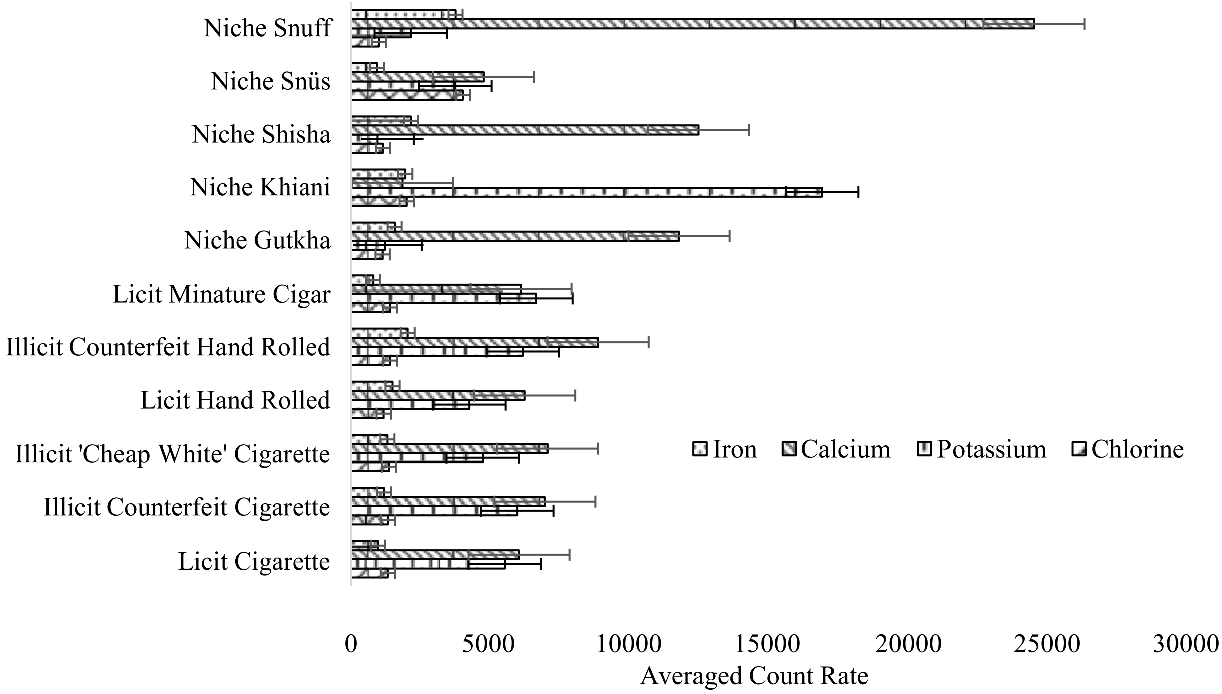
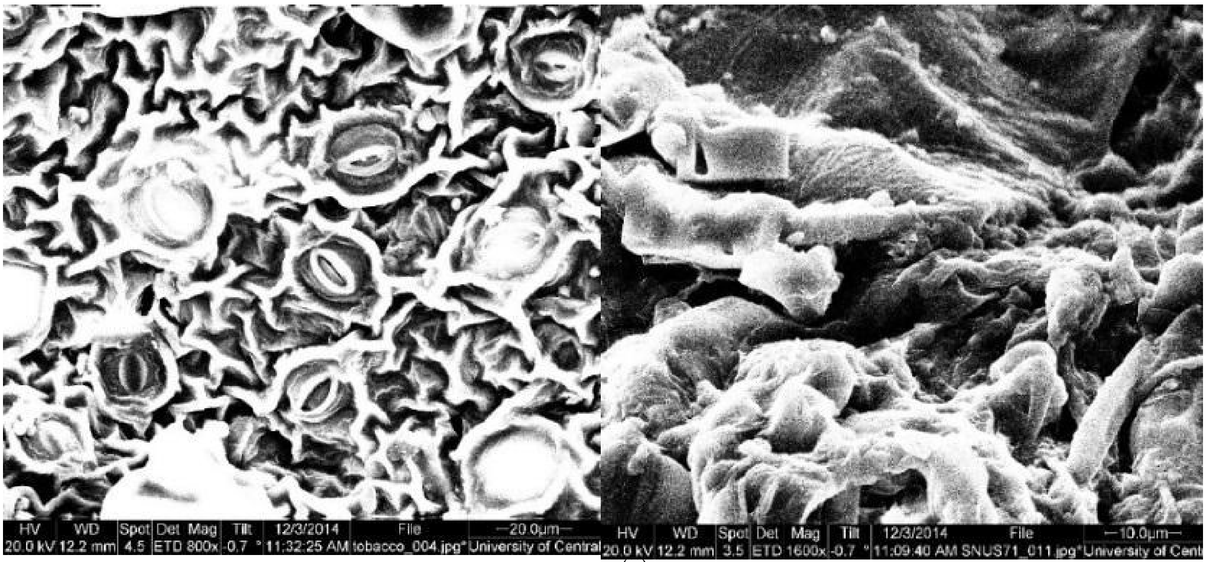


Figure 1. Average count rates, including averaged actual error, for each different type of tobacco provenance based on the four elements that showed the greatest visual variance during XRF analysis.

Figure 2



(i) (ii)

Figure 1. (i) SEM image of whole leaf licit cigarette tobacoo with stomata visible (ii) SEM image of treated niche Swedish Snüs tobacco with visible crystalline structures used for later elemental analysis using EDX.

Figure 3

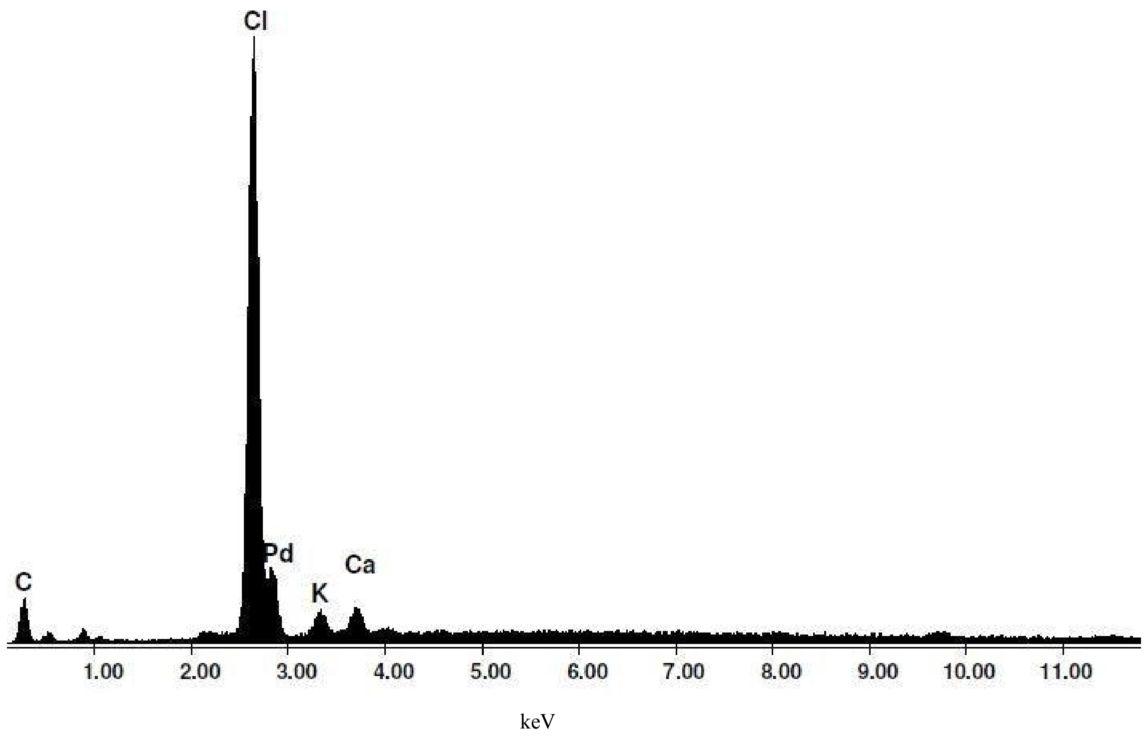
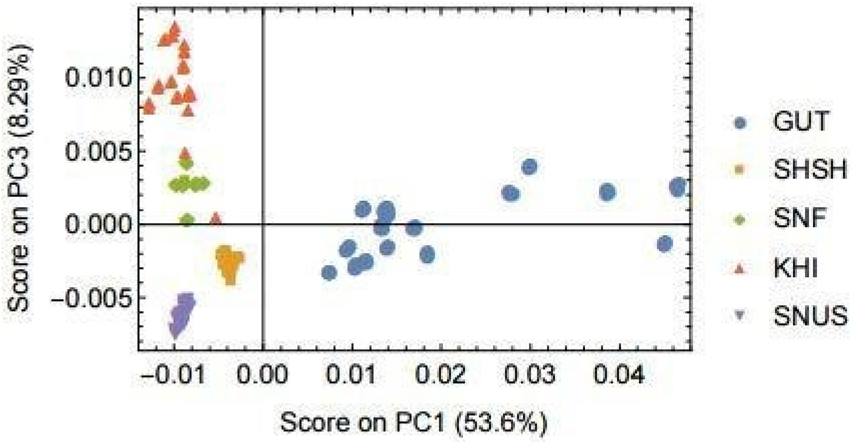


Figure 1. A qualitative EDX elemental profile of crystalline structures found within Swedish Snüs tobacco. Note: the intense Cl peak at 2.65 is thought to be attributed to potassium chloride or calcium chloride salts.

Figure 4

i)



ii)

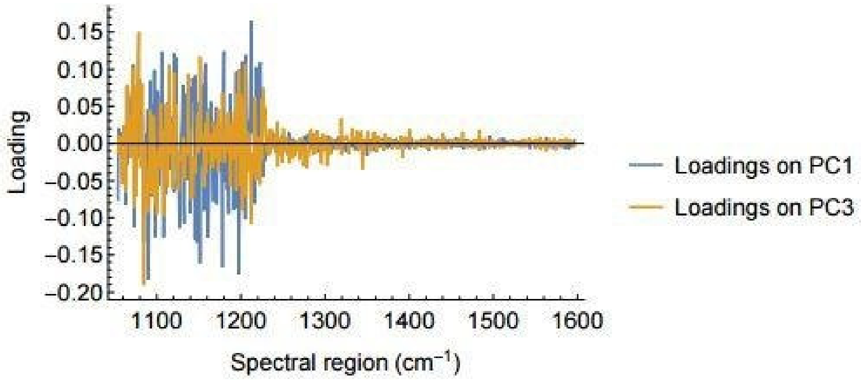


Figure 1. (i) Score and (ii) loading plots of niche tobacco samples, Gutkha, Shisha, Snuff, Snüs and Khaini using PC1/ PC3 loadings of data identified by variable ranking as the tobacco spectral fingerprint region. Plots of PC1 (53% variance) vs PC3 (8%) were used as they show an increased discrimination between the different niche tobacco products when compared to that of PC1 vs PC2 (15% variance).

Table

Table 1. Definition, descriptions, and quantity of different types of tobacco included within this study, all definitions are adapted from the NTPD website [3], http://www.ntpd.org.uk accessed on 08/11/2015 at 23.07.

Tobacco	*Definition and Description*	*Number of Tobacco Samples within study*
Cigarettes	Cigarettes are the most common tobacco product available on the market, made up of flakes of tobacco leaf that have been rolled into a cylindrical shape using a filter and thin filter paper	45
Miniature Cigars	Miniature cigars are roughly the same size as a cigarette, made using large tobacco flakes rolled in whole tobacco leaf to allowing for a slower rate of pyrolysis	2
Hand Rolled	Hand rolled tobacco is usually a blend of several types of tobacco, with thin wiry strands that are rolled using filter paper into a cigarette form or alternatively smoked in a pipe	16
Khaini	Khaini has a predominantly male market within India and Pakistan. Consumed socially, the user combines the Khaini by pressing it into the form of a ball, then places it in the oral cavity where it is then held and sucked occasionally for 10-15 minutes. Khaini contains fragments of leaf material, tobacco, slaked limed paste and areca nut	2
Gutkha	India and Pakistan are the main retailers and consumers of Gutkha products, with a target market of young men and boys. Gutkha is sucked, spat or chewed and typically contains betel nut, catechu, tobacco, lime, saffron and additional flavouring agents specific to the brand	4
Snuff	Fire cured tobacco, more commonly known as snuff, is found in a dry powdered form with less than 10% moisture content which is then either sniffed or held in the oral cavity. Snuff is a product typically found within the U.K, USA, India and Sweden	2
Snüs	Predominantly produced in Sweden on a large scale, Snüs is found in either loose or pouched form with a typical portion being between 0.5g-1.0g. The pouch or loose tobacco is held in between the wet membrane of the gum and	5

	cheek to allow for rapid absorption of constituents into the blood stream. The tobacco is finely ground, dried and mixed with aromatic substances, salts, humidifying agents such as Sodium Carbonate, additional nicotine and water	
Shisha	Shisha or Water pipe tobacco, the composition of which varies and is typically found to have thick almost bark like fragments of tobacco leaf mixed with artificial flavourings. Additional nicotine and aromatic compounds are present resulting in a sticky oily residue that is used to give a specific desired fragrance to the tobacco. Shisha tobacco is marketed based on flavour/fragrance and is typically produced in North African countries, Eastern Europe and Southern Asia	3

Table2

Table 1. Specific absorbances identified after data pre-processing and multivariate data analysis using variable ranking, highlighting the tobacco spectral fingerprint region.

Functional Group	***Absorptions***	***Vibrational Mode***
C-O	1050-1150 cm^{-1}	Carbonyl Stretch
-C-H	1350-1480 cm^{-1}	Alkane Bending
C=O	1600-1700 cm^{-1}	Amide I Stretching
C-H	2850-3000 cm^{-1}	Alkane Stretch
O-H	3200-3600 cm^{-1}	Alcohol Stretch

Supplementary Information- Sample List

The table below shows the full sample list of tobaccos utilised within this study, including packet number and sample provenance.

Sample	*Brand*	*Licit*	*Illicit*	*Niche*	*Country of origin*	*Tobacco type*	*Quantity*
1	Richman		X		Unknown	Cigarettes	4
2	Marlboro	X			Spain	Cigarettes	1
3	New Line		X		Russia	Cigarettes	4
4	Brendal		X		Unknown	Cigarettes	4
5	Jin Ling		X		Russia	Cigarettes	1
6	Golden Virginia		X		Germany	Hand Rolled	2
7	Palace	X			Spain	Cigarettes	1
8	Master		X		Arabic	Cigarettes	1
9	Marlason		X		Unknown	Cigarettes	1
10	Tulsi Royal			X	India/Pakistan	Gutkha	5
11	Marlboro				United Kingdom	Cigarettes	1
12	Marhaba Molasses and Apple			X	India	Shisha	1
13	Dubai Tobacco Cappucino			X	United Arab Emirates	Shisha	1
14	Unlabelled Snuff			X	Unknown	Snuff	1
15	Black Snuff			X	Unknown	Snuff	1
16	Marble		X		Switzerland	Cigarettes	1
17	Richmond	X			Spain	Cigarettes	2
18	Benson & Hedges	X			Polish	Cigarettes	2
19	John Player		X		Pakistan	Cigarettes	2
20	Amber Leaf	X			Unknown	Hand Rolled	2
21	Castella	X			Unknown	Cigarettes	2
22	Castella Classic	X			Unknown	Cigarettes	4
23	Richmond	X			Unknown	Cigarettes	1
24	Beaumont	X			Unknown	Cigarettes	1
25	Park Drive	X			Unknown	Cigarettes	1
26	King Edward	X			Unknown	Cigarettes	1
27	Castella Miniatures	X			Unknown	Minature Cigar	1
28	Henri Wintermans	X			Holland	Minature Cigar	1
29	Regal				Unknown	Cigarettes	2
30	Regal				Unknown	Cigarettes	2
31	Lambert & Butler	X			Spain	Cigarettes	2
32	Richman		X		Unknown	Cigarettes	2
33	Royals	X			Unknown	Cigarettes	2
34	Consulate menthol	X			Unknown	Cigarettes	2

35	Golden Virginia	X			Germany	Hand Rolled	2
36	Superkings		X		Unknown	Cigarettes	3
37	Essential		X		Spain	Cigarettes	3
38	Bon International		X		Unknown	Cigarettes	3
39	Raquel		X		Unknown	Cigarettes	2
40	Golden Virginia	X			Spain	Hand Rolled	2
41	Lambert & Butler	X			Spain	Cigarettes	2
42	Silk cut	X			Spain	Cigarettes	2
43	Silk cut	X			Spain	Cigarettes	2
44	Amber Leaf	X			Spain	Hand Rolled	2
45	L&M Blue label	X			Arabic	Cigarettes	3
46	Amber Leaf		X		Germany	Hand Rolled	2
47	Raquel		X		Unknown	Cigarettes	2
48	Jin Ling		X		Russia	Cigarettes	2
49	Gold Classic		X		Unknown	Cigarettes	2
50	Match 444		X		Unknown	Cigarettes	2
51	Sovereign		X		Russia	Cigarettes	2
52	Palace		X		Spain	Cigarettes	2
53	Golden Virginia	X			Netherlands	Hand Rolled	2
54	Golden Virginia		X		Unknown	Hand Rolled	1
55	Golden Virginia		X		Unknown	Hand Rolled	4
56	Lilja Mazaya Apple			X	Arabic	Shisha	2
57	Star premium			X	India	Gutkha	6
58	Mirage Lime mixed			X	India	Khiani	3
59	RMD			X	India/ Pakistan	Gutkha	4
60	GOA 10000			X	India/Pakistan	Gutkha	7
61	Miraj			X	India/ Pakistan	Khiani	6
62	Lossnus Grov			X	Sweden	Snüs	1
63	L.D Los			X	Sweden	Snüs	1
64	G'R Goteborgs rape			X	Sweden	Snüs	1
65	Gerneral classic white			X	Sweden	Snüs	1
66	Kaliber original			X	Sweden	Snüs	1
67	Scaferlati Caporal	X			France	Hand Rolled	1
68	Gauloies Brunes	X			Belgium	Cigarettes	1
69	Old Holborn	X			France	Hand Rolled	1
70	Winston Classic	X			France	Hand Rolled	1
71	Amandis	X			Belgium	Hand Rolled	1
72	Gouloises Brunes	X			France	Hand Rolled	1
73	Regal king size	X			United Kingdom	Cigarettes	1
74	Amber leaf	X			United Kingdom	Hand Rolled	1
75	Marlboro	X			United Kingdom	Cigarettes	1
76	Silk cut	X			United Kingdom	Cigarettes	1
77	Lambert & Butler	X			United Kingdom	Cigarettes	1
78	Benson & Hedges	X			United Kingdom	Cigarettes	1

79	Golden Virginia	X	United Kingdom	Hand Rolled	1

Supplementary Information- PCA Plots

PCA plots have been included in the supplementary information to show PC1 Vs PC3 plots showing better discrimination between loadings. PCA is an unsupervised multivariate analysis technique that provides eigenvectors (PCs) that describe the variance within the dataset. It is correct to state that PC2 will have a higher eigenvalue but the ability to discriminate will depend upon the dataset analysed. PC1 vs PC2 does not necessarily lead to the best discrimination if the feature which most accurately enables discrimination of the data is small then this feature may appear in higher PCs.

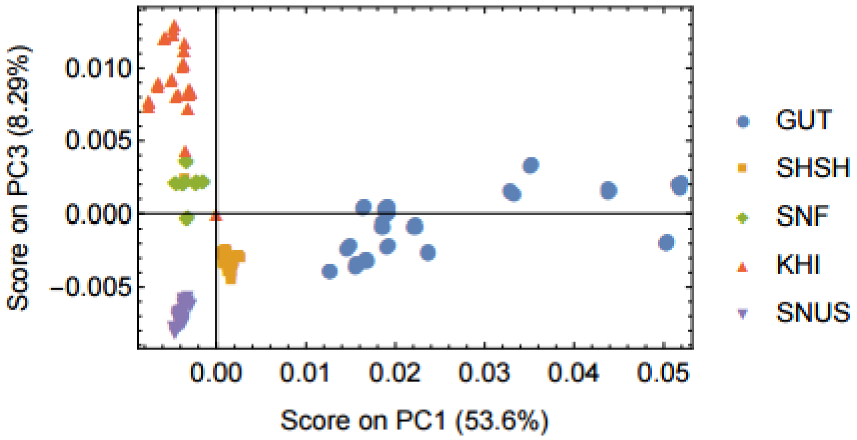


Figure 1 (i) Score plots of niche tobacco samples, Gutkha, Shisha, Snuff, Snüs and Khaini using PC1/ PC3 loadings of data identified by variable ranking as the tobacco spectral fingerprint region. Plots of PC1 (53% variance) vs PC3 (8%).

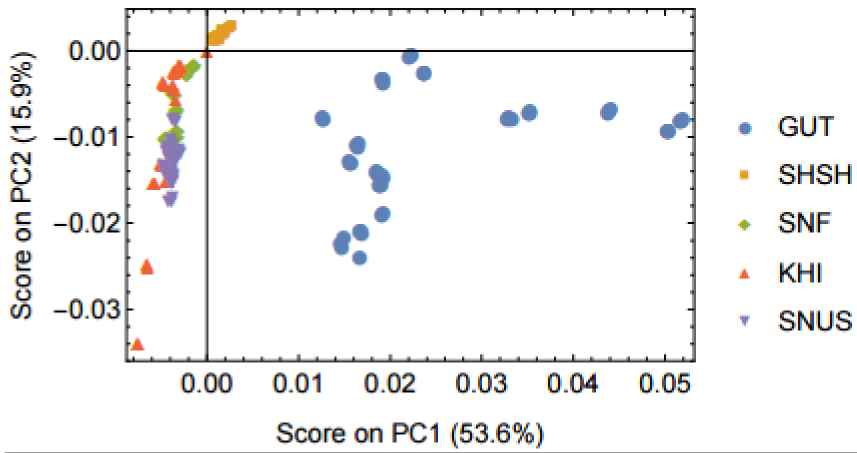


Figure 2 Score plot of niche tobacco samples, Gutkha, Shisha, Snuff, Snüs and Khaini using PC1/ PC2 loadings of data identified by variable ranking as the tobacco spectral fingerprint region. Plots of PC1 (53.6% variance) vs PC2 (15.9%)

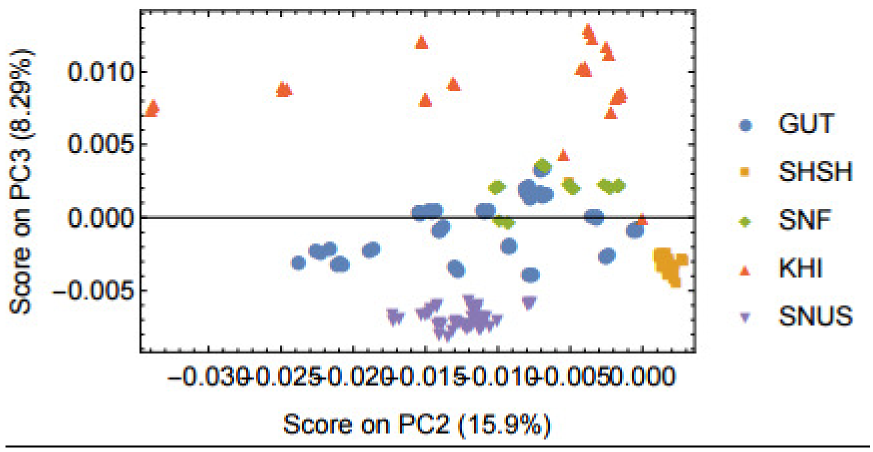


Figure 3 Score plot of niche tobacco samples, Gutkha, Shisha, Snuff, Snüs and Khaini using PC2/ PC3 loadings of data identified by variable ranking as the tobacco spectral fingerprint region. Plots of PC2 (15.9% variance) vs PC3 (8.29%)