

Electronic supplementary information: Combining near-infrared (NIR) analysis and modelling as a fast and reliable method to determine the authenticity of agarwood (*Aquilaria* spp.)

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Analysis methods

Microscopic

Wood fragments were softened in boiling water. Thin transverse (TS), tangential longitudinal (TLS) and radial longitudinal sections (RLS) were cut using a microtome blade. The thin sections were mounted in glycerol on glass microscope slides, covered with a cover slip and examined with a light microscope.

LC-MS

Sample preparation

50 mg of each of the commercial and authentic agarwood samples were extracted with 1 mL of pure HPLC grade methanol for 24 hrs in the darkness. After extraction, samples were filtered and placed in 2 mL glass vials for LC-MS analysis.

LC-MS parameters

LC-MS analyses were performed on a Vanquish UHPLC system (Thermo Scientific, Waltham, MA, USA) coupled to a 100 Hz photodiode array detector (PDA) and an Orbitrap Fusion Tribrid (Thermo Scientific) high-resolution tandem mass spectrometer. Chromatographic separation of plant extracts (5 μ L) was performed on a Luna C18 column (150 mm \times 3 mm i.d., 3 μ m, Phenomenex, Torrance, CA, USA) using a mobile phase gradient of 0:90:10 to 90:0:10 ([MeOH (A): water (C): acetonitrile +1% formic acid (D)]) over 60 min. Then, 90% A was held for 10 min and then returned to initial conditions over 5 min at 30 $^{\circ}$ C (flow rate: 400 μ L/min). UV detection was recorded between 210 and 550 nm.

Mass spectrometry detection was performed in both positive and negative ionization modes using the full scan and data dependent MS2 and MS3 acquisition modes. Total ion current (TIC) chromatograms were obtained over the range of 125–1800 m/z using a spray voltage of +3.5 and –2.5 kV for the positive and negative ionization modes, respectively. Four different scan events were recorded for each ionization mode as follows: (1) full scan, (2) MS2 of the most intense ion in Scan Event 1, (3) MS3 of the most intense ion in Scan Event 2, and (4) MS3 of the second most intense ion in Scan Event 2. Additional parameters for the mass spectrometer included full scan resolution, 60,000 FWHM; capillary temperature, 350 $^{\circ}$ C; ion transfer tube temperature, 325 $^{\circ}$ C; RF lens (%), 50; automatic gain control (AGC) target, 4.0×10^5 (full scan) and 1.0×10^4 (MSn); intensity threshold, 1.0×10^4 ; CID collision energy, 35 eV; activation Q, 0.25; isolation window (m/z), 4. Nitrogen was used as the drying, nebulizer, and fragmentation gas.

The identification of compounds was performed by comparing the monoisotopic mass and MS2 spectra of the experimental data with data from our in-house library of MS2 spectra (Nist) and data reported in the literature for Agarwood.

Software parameters

Data pre-processing: noise weighting filter, MSC normalisation

Algorithm: SIMCA

Score expansion: 1.5

Residual expansion: 0.72

Factors: maximum 100, minimum 0

RT: 0.00 - 35.03

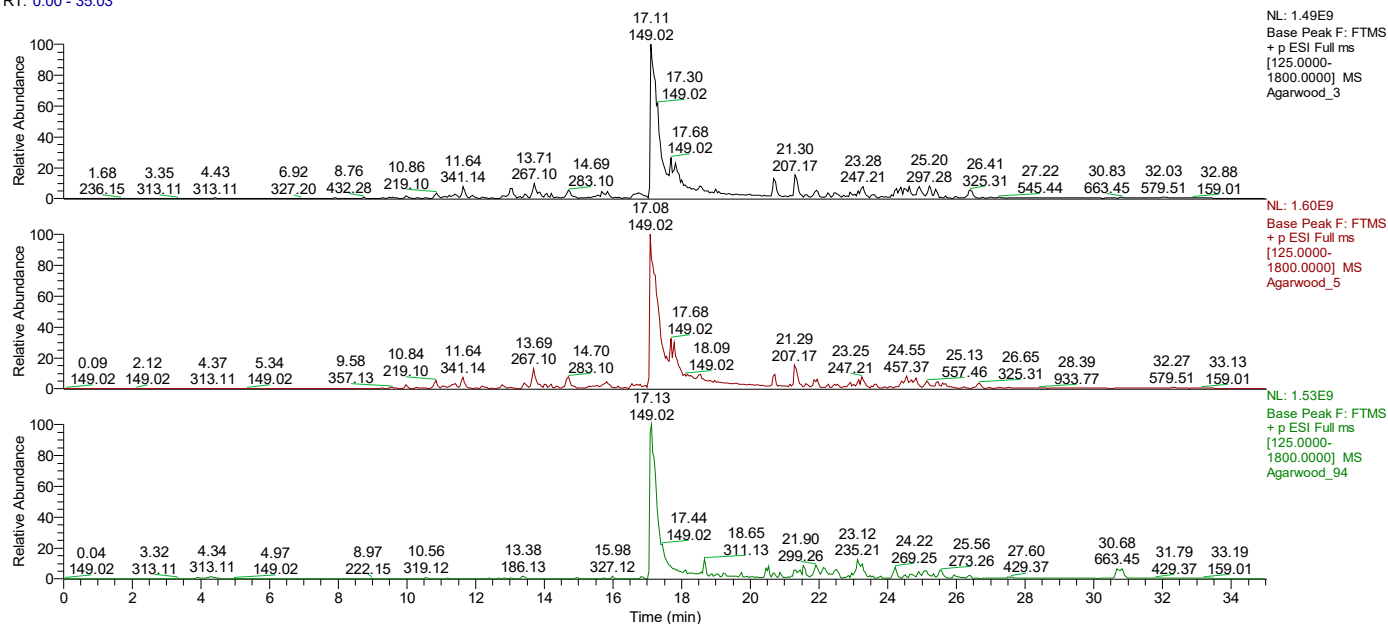


Fig. S1 Positive mode LC-MS chromatograms of three representative agarwood samples

RT: 0.00 - 35.03

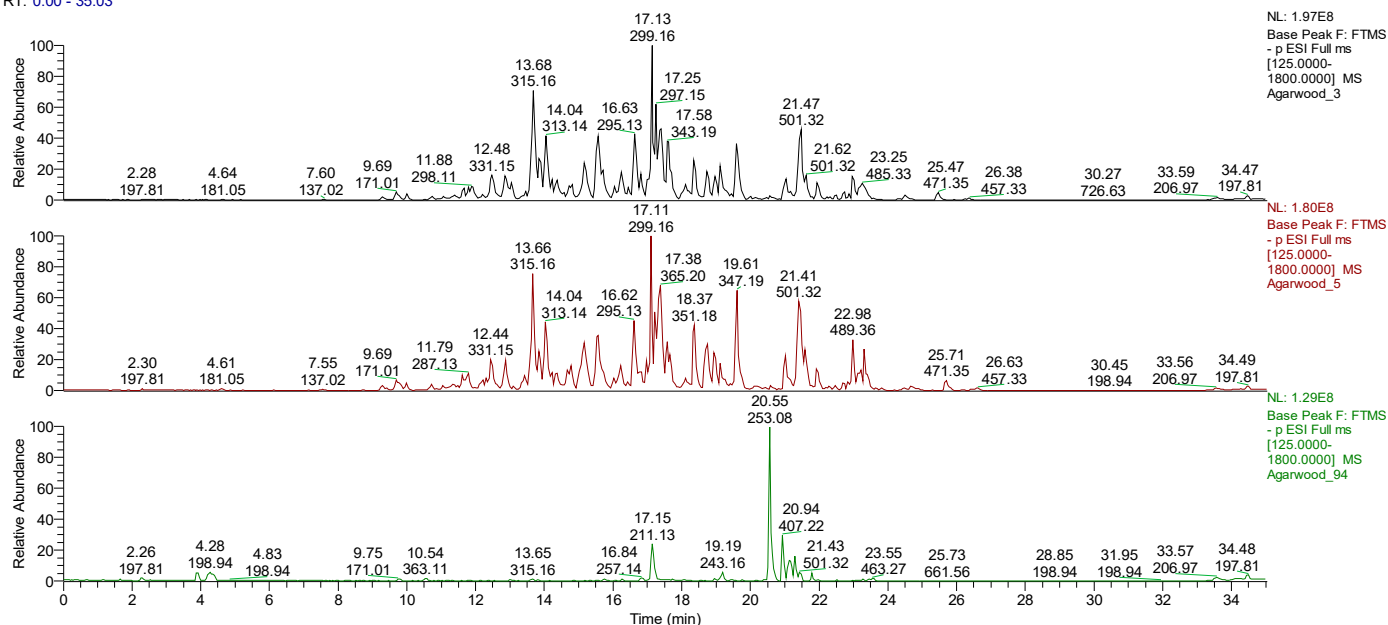


Fig. S2 Negative mode LC-MS chromatograms of three representative agarwood samples

Table S1 Samples included in study

Number	Format	Morphological	Chemical	Other notes
1	crumbs	Not agarwood		not identified
2	crumbs	Not agarwood		not identified
3	powder	<i>No structure</i>	Substituted	
4	chips	Authentic		
5	powder	<i>No structure</i>	Substituted	
6	chips	Authentic		
13	chips	Not agarwood		Eucalyptus
7	chips	Authentic		
8	small flakes	Authentic		
9	small flakes + powder	Not agarwood		Lauraceae
10	chips + resinous powder	Authentic		
11	chips + resinous powder	Authentic		
12	flakes + resinous powder	Authentic		
14	chips	Authentic		
15	chips	Authentic		
16	chips	Authentic		
17	chips	Authentic		
18	chips	Authentic		
19	chips + resinous powder	Authentic		
20	chips + resinous powder	Authentic		
21	chips + resinous powder	Authentic		
22	chips	Not agarwood		not identified
23	chips	Not agarwood		not identified
24	large piece	Not agarwood		not identified
25	chips + resinous powder	Authentic		
26	chips + resinous powder	Authentic		
27	chips	Authentic		
28	chips	Authentic		
29	chips	Authentic		
30	chips	Authentic		
31	resin chunks + wood flakes	Authentic		
32	flakes	Authentic		
33	chips	Authentic		
34	resin crumbs + wood flakes	Authentic		
35	chips	Authentic		
36	chips (a) chips (b)	Authentic		
37	chips	Authentic		
38	powdery chunks	Not agarwood		corms, possibly Iridaceae
39	powder	Not agarwood		Daniella
40	<i>resinous chunks</i>	<i>Not agarwood</i>		<i>no plant material</i>
41	bright yellow resin chunk	Not agarwood		possibly Picea
42	small flakes	Not agarwood		Pineus
43	small resinous chunks	Not agarwood		corms, possibly Iridaceae
44	oily powder	Not agarwood		Daniella
45	powdery chunks	Not agarwood		corms, possibly Iridaceae
46	<i>resinous chunks</i>	<i>Not agarwood</i>		<i>no plant material</i>
47	<i>resin</i>	<i>Not agarwood</i>		<i>no plant material</i>
48	<i>resinous chunks</i>	<i>Not agarwood</i>		<i>no plant material</i>

49	resin + wood flakes	Not agarwood		Santalum
50	oily black chips	Authentic		
51	oily black chips	Authentic		
52	resin + wood flakes	Not agarwood		Santalum
53	chips	Authentic		
54	chips	Authentic		
55	chips	Authentic		
56	chips	Authentic		
57	chips	Authentic		
58	chips	Authentic		
59	chips		Authentic	
60	chips		Authentic	
61	chips		Authentic	
62	oily flakes		Substituted	
63	chips		Diluted	
64	chips		Diluted	
65	chips		Diluted	
66	chips		Diluted	
67	powder		Authentic	
68	powder		Authentic	
69	chips		Substituted	
70	chips		Diluted	
71	powder		Diluted	
72	powder		Authentic	
73	chips		Substituted	
74	powder		Authentic	
75	chips		Substituted	
76	chips		Substituted	
77	chips	Authentic		
78	chips	Authentic		
79	flakes	Not agarwood		Santalum
80	flakes	Not agarwood		Santalum
81	chips	Authentic		
82	chips	Not agarwood		Santalum
83	<i>resin lumps</i>	<i>No plant material</i>		
93	wood flakes + resin chunks	Authentic		
94	oily flakes	<i>No structure</i>	Authentic	

NIR data

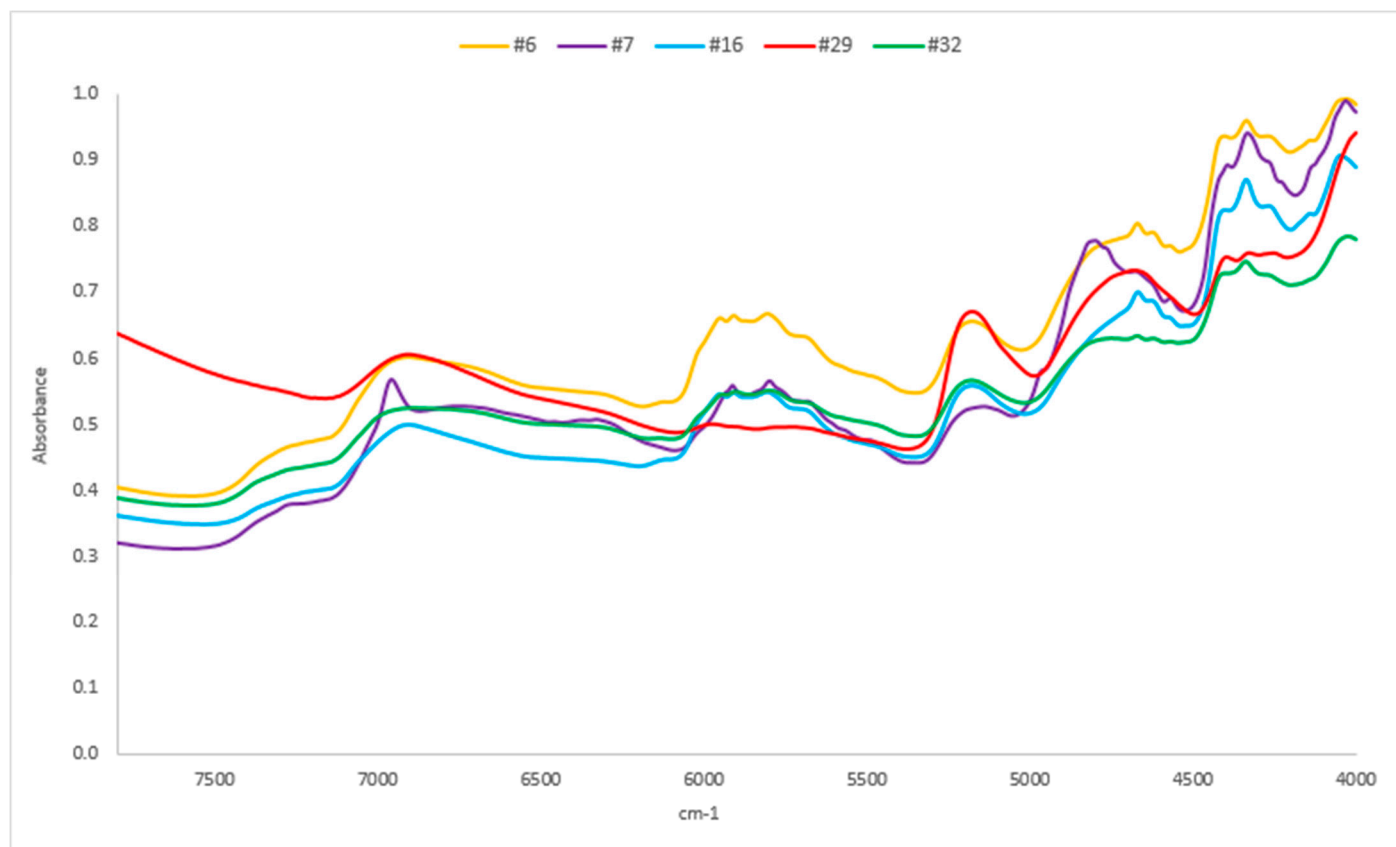


Fig. S3 Five representative NIR spectra of 'Authentic' group

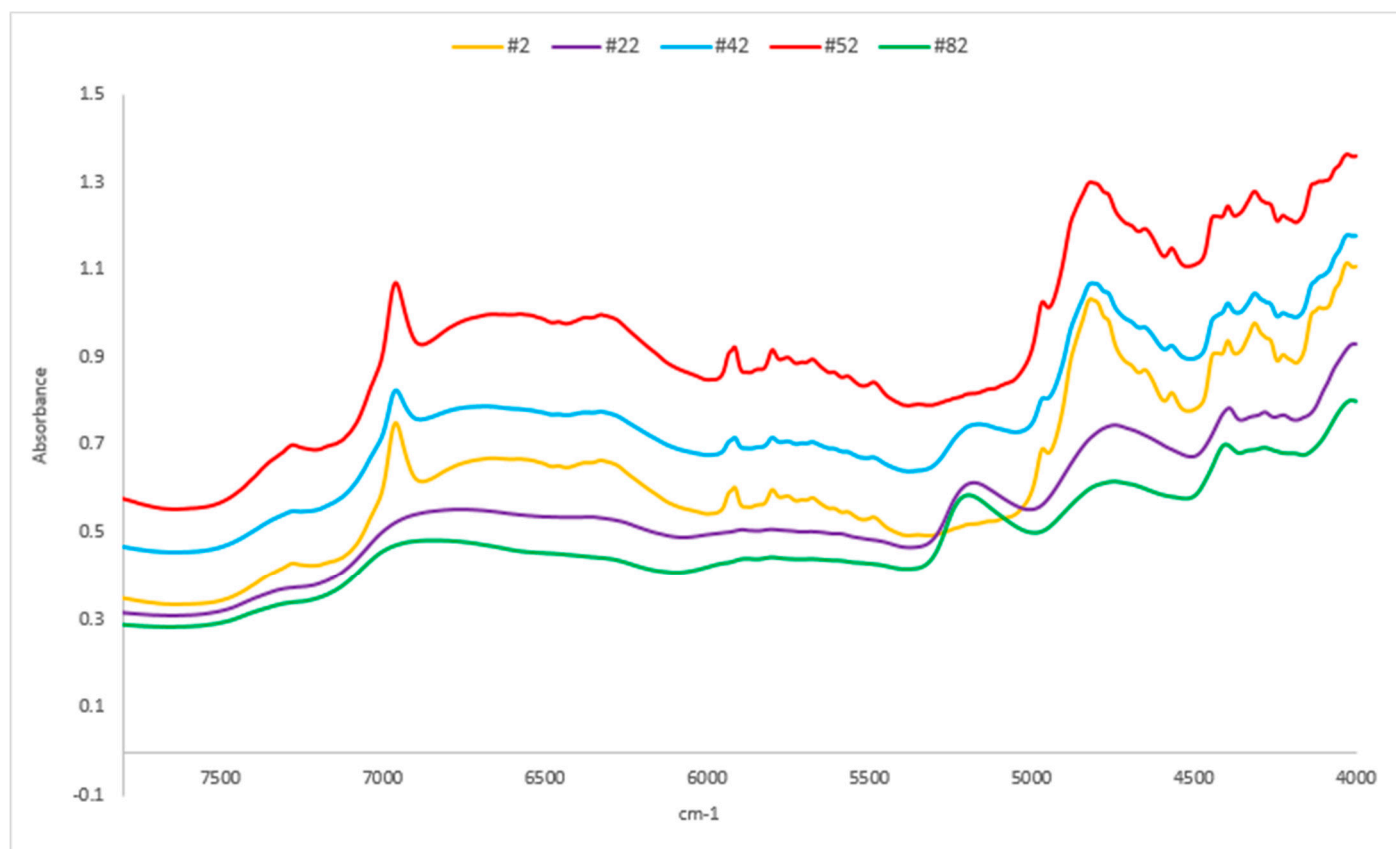


Fig. S4 Five representative NIR spectra of 'Substituted' group

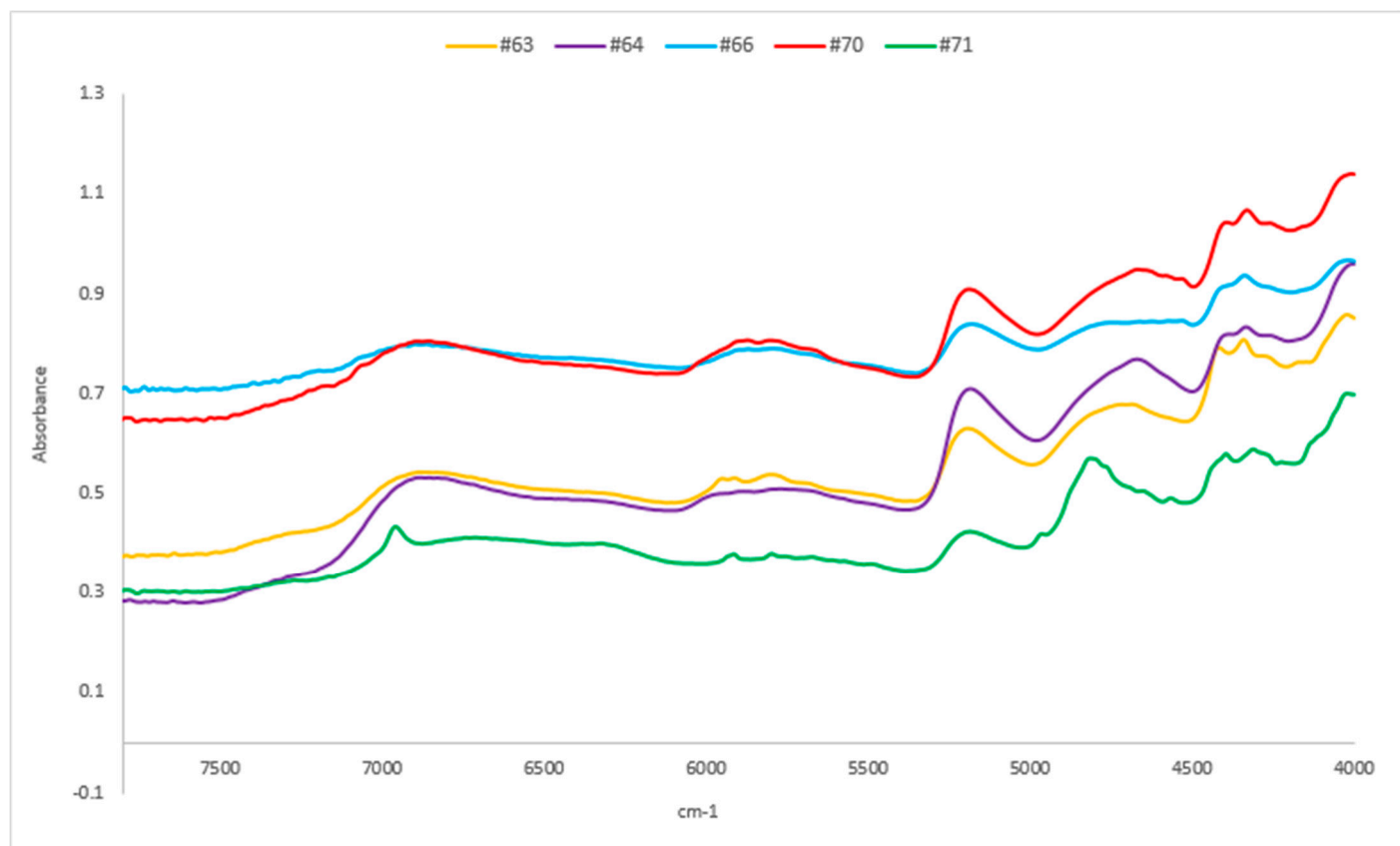


Fig. S5 Five representative NIR spectra of 'Adulterated' group

Model

Effect of sample washing

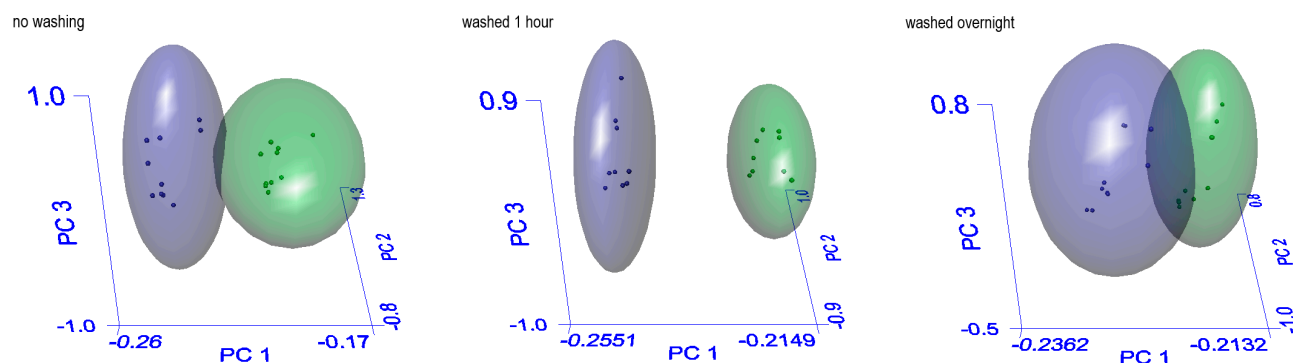


Fig. S6 Model using data from washed, unwashed, and over-washed samples. Blue = non-authentic. Green = authentic.

Effect of baseline correction

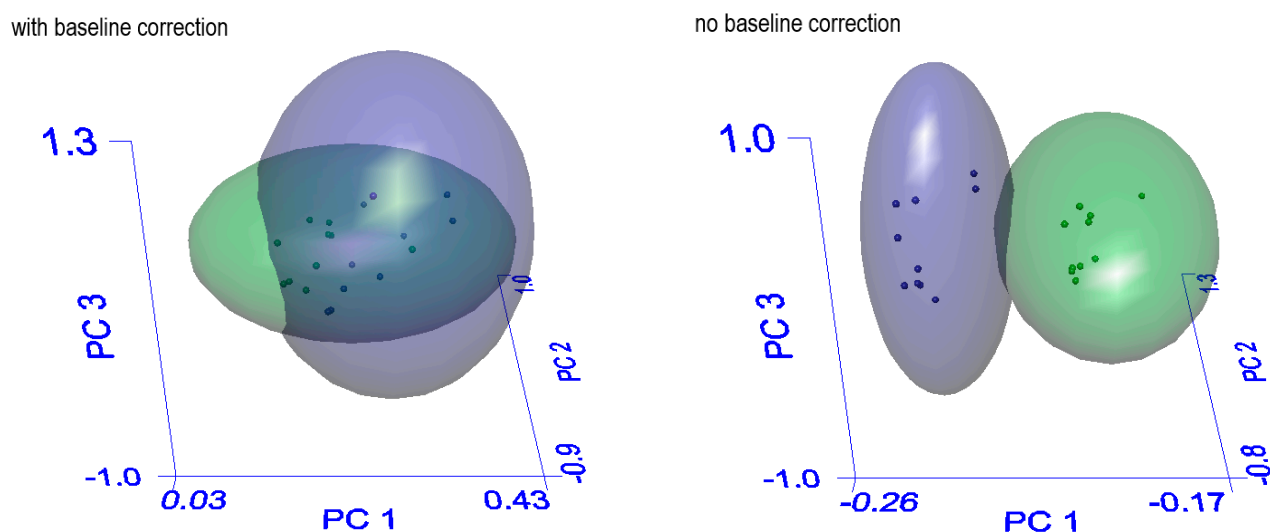


Fig. S7 Model with and without baseline correction. Blue = non-authentic. Green = authentic.

Table S2 Validation results

Sample ID	Class	Assignment	Result	Material distance ratio	total	Material distance	residual
1	Non-authentic	Non-authentic	Passed	0.9897		1.4256	
38	Non-authentic	Non-authentic	Passed	0.7115		1.0249	
44	Non-authentic	Not assigned	Failed	1.0611		1.5284	
62	Non-authentic	Not assigned	Failed	1.4327		2.0637	
69	Non-authentic	Not assigned	Failed	1.2722		1.8326	
8	Authentic	Not assigned	Failed	1.5044		2.0739	
81	Authentic	Not assigned	Failed	1.6635		2.2933	
12	Authentic	Non-authentic	Failed	1.3943		1.9221	
14	Authentic	Not assigned	Failed	1.3708		1.8897	
16	Authentic	Not assigned	Failed	2.2241		3.0661	