

Supplementary Information

Paper fragments from the Tibetan Samye Monastery: Clues for an unusual sizing recipe implying wheat starch and milk in early Tibetan papermaking

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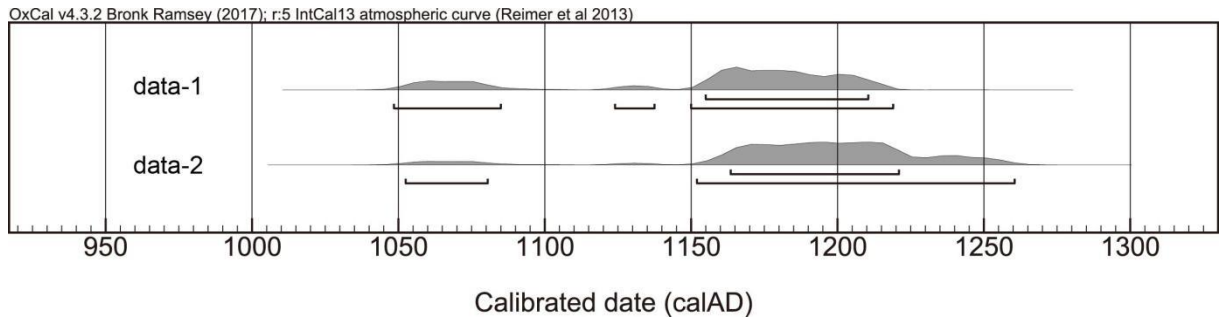


Figure S1. ^{14}C radiocarbon dating result of the ancient Tibetan fragment measured by AMS facility in Nagoya University (data-1) and Beta Analytic Radiocarbon Dating Laboratory (data-2).

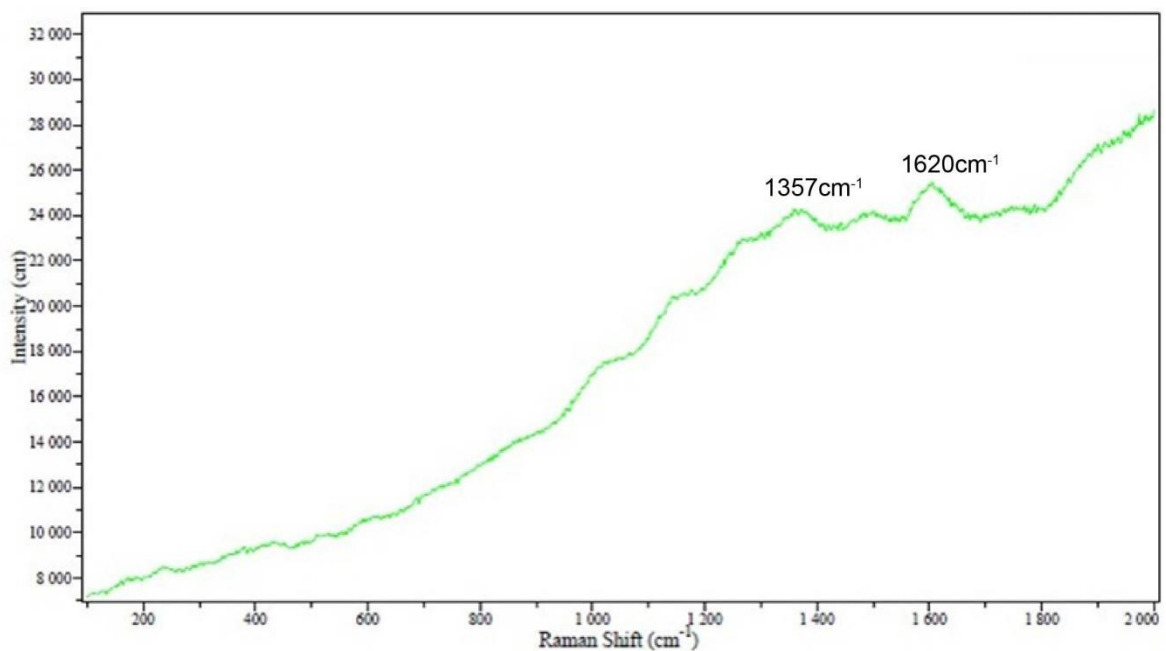


Figure S2. Raman spectrum of the ink present in the ancient Tibetan fragment. Raman analysis was performed with a Laser Micro-Raman Spectrometer (JY XploRA, France). The sample excitation was performed with a 532 nm laser and focused through a 50 \times objective lens. Spectra of the samples were recorded with a resolution of 2 cm^{-1} and three accumulations of 20 s each in the range 100–2000 cm^{-1} .

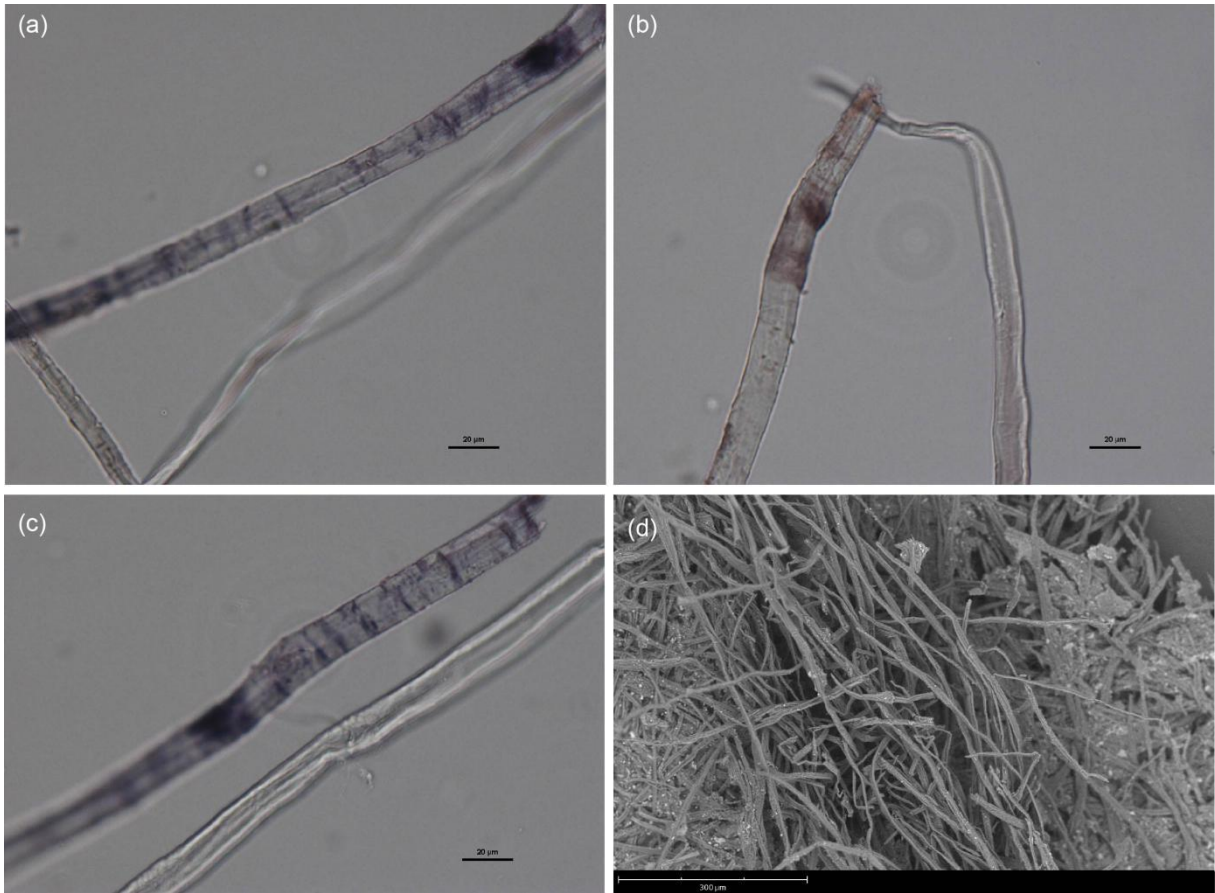


Fig. S3 the microscopic observation of paper fiber from the ancient Tibetan paper fragment.

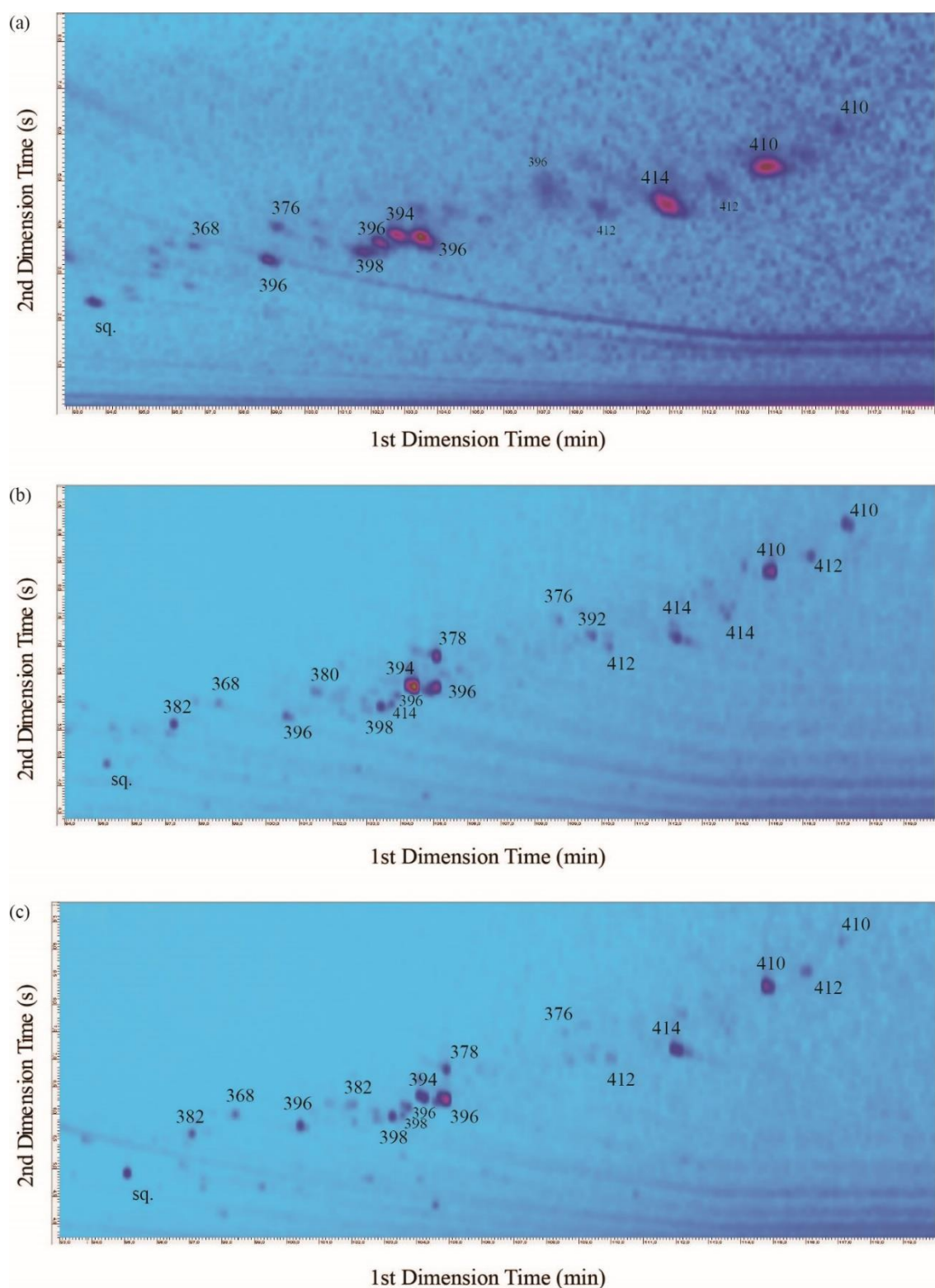


Figure S4. Comparison of the ROI for marker plants obtained from the Py-GCxGC/MS analysis of (a) the ancient Tibetan paper sample, (b) a reference mitsumata paper sample, (c) a reference gampi paper sample. Labels in the figure correspond to the expected molecular weights (MW) of each marker deduced from their corresponding mass spectra. The scales of retention times are different between (a) and (b)-(c) due to different settings of columns (loop length and second dimension column length).

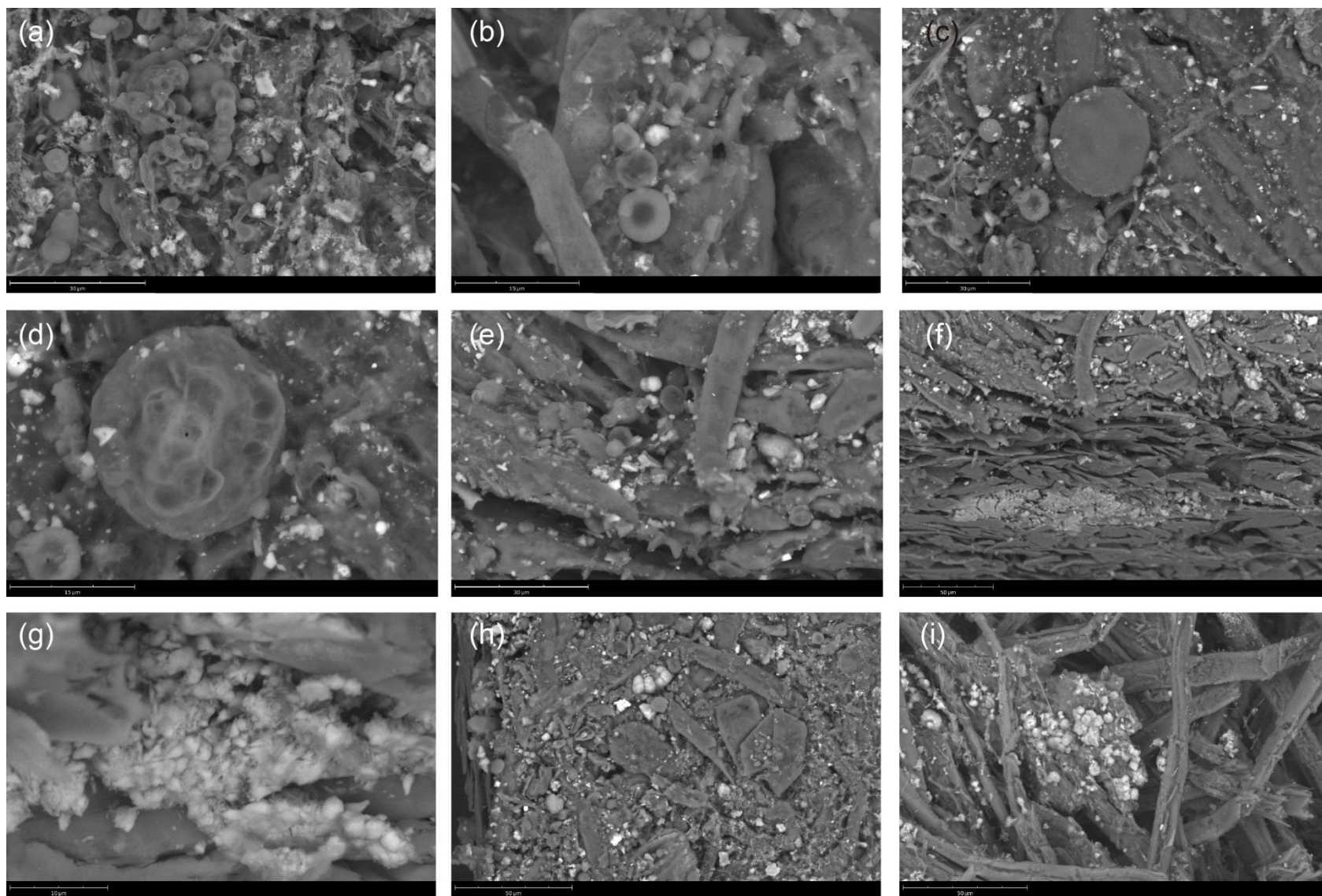


Figure.S5 the SEM observation of the Tibetan Samye Monastery paper fragment.

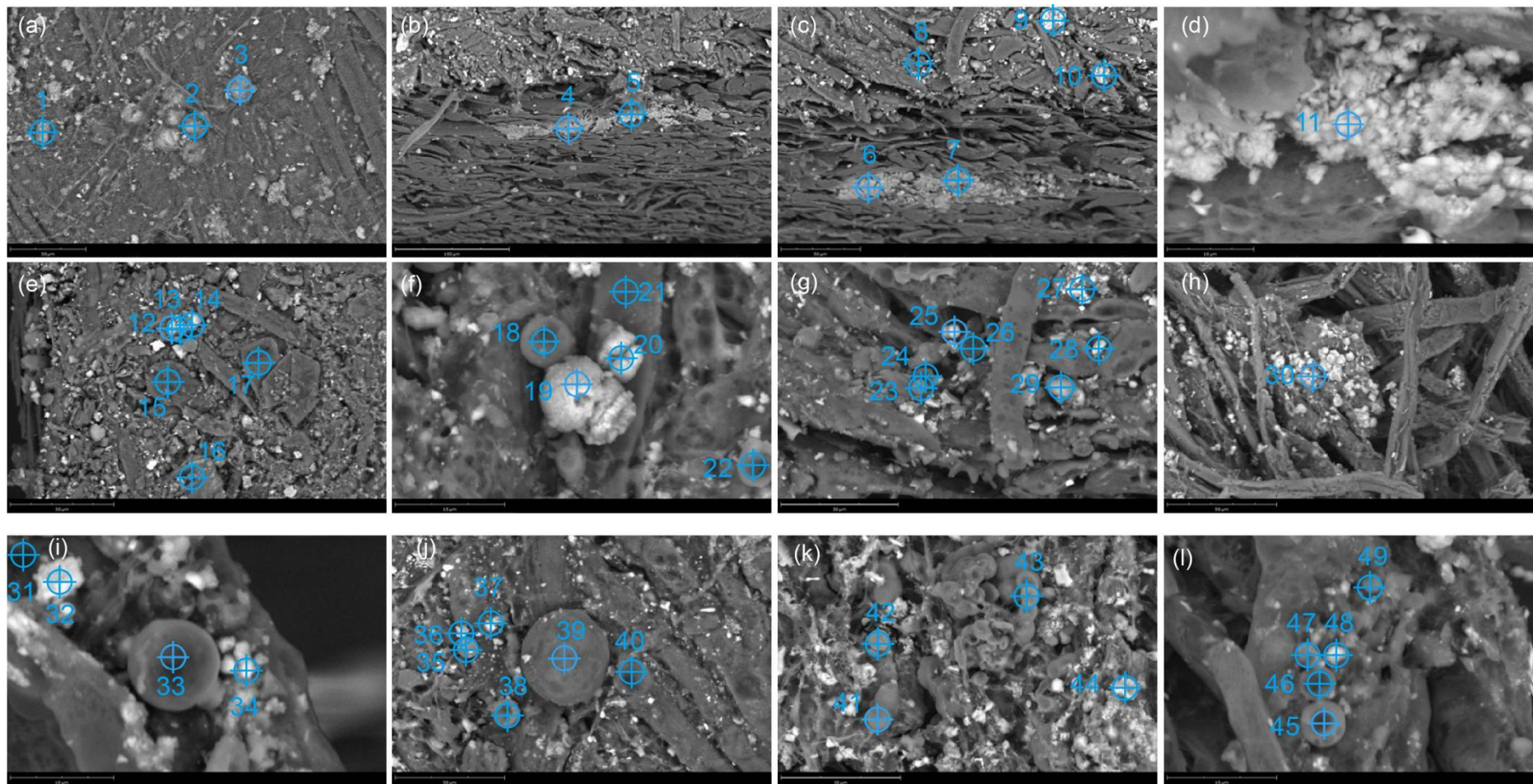


Figure.S6 the EDS elemental analysis results of the Tibetan Samye Monastery paper fragment.

Table S1 elemental analysis showing different elements and corresponding ratio (Weight Conc.). For the point number, refer to Fig.S6.

Point	C	O	Ca	Mg	K	Na	Cl	Al	P	S	Cu	Si	N	Mn
1	30.26	44.79	16.93	3.11	2.3	–	0.4	–	1.4	0.6	0.2	–	–	–
2	22.9	45.5	25.4	5.8	–	–	0.3	–	–	–	0.1	–	–	–
3	25	37.6	26.1	4.2	3	–	0.4	–	1.8	–	0.4	–	–	–
4	29.72	38.05	22.09	5.22	2.41	–	0.4	–	1.41	0.6	0.1	–	–	–
5	38.72	35.01	16.45	3.71	3.51	–	0.9	0.7	0.9	–	0.1	–	–	–
6	28.97	44.06	21.08	5.29	–	–	0.4	–	–	–	0.2	–	–	–
7	29.5	40.4	24.4	4.9	–	–	0.7	–	–	–	0.1	–	–	–
8	54.8	27.9	–	–	3.2	0.9	1.4	–	0.6	0.6	0	0.7	9.9	–
9	27.4	37	28.9	1.5	2.2	–	0.5	0.7	–	0.8	0.1	0.9	–	–
10	22.72	34.83	33.63	2.5	2.5	–	0.5	–	–	0.5	0	–	–	2
11	27.4	33	29.6	3.4	3	–	0.6	0.8	1.5	0.6	0.1	–	–	–
12	24.6	35.84	31.53	3.11	2.31	0.5	0.6	–	–	–	0	–	–	1.51
13	23.85	39.78	29.46	3.11	2.1	–	0.5	–	–	–	0	–	–	1.2
14	25.9	38.8	30.9	3.8	–	–	0.6	–	–	–	0	–	–	–
15	60	25	2.8	–	4.9	1.1	3.3	0.6	–	1	0	1.3	–	–
16	60.84	26.1	3.11	0.9	5.42	1.41	1.61	–	–	0.5	0.1	–	–	–
17	60.7	27.6	1.5	–	6.2	0.8	1.9	0.6	–	–	0	0.7	–	–
18	57.13	26	3.82	0.7	8.63	1	1.91	–	–	0.8	0	–	–	–
19	23.82	41.74	26.93	4.1	–	–	0.4	–	–	–	0.1	–	2.9	–
20	30.49	30.59	30.29	3.01	2.81	–	0.9	–	–	0.8	0.1	–	–	–
21	62.06	25.13	1.61	–	8.38	1.01	1.82	–	–	–	0	–	–	–
22	58.2	28.3	3.2	0.9	5.5	0.7	1.7	0.6	–	–	0	0.9	–	–
23	63.5	25.6	1.5	–	4	1.3	1.8	–	–	0.7	0	1.6	–	–
24	52.07	31.24	3.44	–	8.19	–	2.12	1.11	–	–	0	1.82	–	–
25	26.23	42.31	24.92	3.02	–	–	0.5	–	–	–	0.1	–	2.91	–
26	57.52	32.39	2.32	–	5.25	0.71	1.72	–	–	–	0.1	–	–	–
27	27.4	37	28.9	1.5	2.2	–	0.5	0.7	–	0.8	0.1	0.9	–	–
28	28.14	43.52	–	–	–	5.03	0.2	7.64	–	–	0	14.37	1.11	–

29	29.1	28	38.6	3.4	-	-	0.8	-	-	-	0.1	-	-	-
30	22.9	45.3	23.5	4.3	2	0.8	0.6	-	0.6	-	0	-	-	-
31	69	23.8	-	-	4.2	1.6	1.4	-	-	-	0	-	-	-
32	29.13	46.98	20.56	-	2.22	-	1.11	-	-	-	0	-	-	-
33	59.98	26.31	2.62	-	6.85	0.91	2.72	0.5	-	-	0.1	-	-	-
34	45.58	23.69	18.57	2.71	4.32	1	1.31	1.41	-	-	0.1	1.31	-	-
35	57.66	31.43	1.1	0.6	-	1.7	1.6	-	-	-	0	0.6	5.31	-
36	29.33	43.64	3	-	0.8	3.8	0.3	7.91	-	-	0	11.21	-	-
37	47.72	11.25	-	-	6.89	12.97	20.16	-	-	-	0	-	-	-
38	60.04	27.51	1.1	0.7	5.32	2.41	2.91	-	-	-	0	-	-	-
39	52.81	27.15	1.6	-	9.22	4.21	5.01	-	-	-	0	-	-	-
40	42.61	31.76	12.26	0.6	8.34	1.01	2.71	-	-	0.6	0.1	-	-	-
41	62.16	28.93	2.2	1.1	2.8	1.3	0.9	0.6	-	-	0	-	-	-
42	54.23	32.8	4.12	1.31	3.62	1.51	1.61	0.8	-	-	0	-	-	-
43	50.35	32.6	4.91	1.5	5.62	1.5	2.21	1.2	-	-	0.1	-	-	-
44	36.75	37.65	16.87	3.41	3.11	0.9	0.6	0.7	-	-	0	-	-	-
45	56.66	25.63	2.5	0.6	8.61	1.4	3.1	0.8	-	-	0	0.7	-	-
46	58.23	21.69	2.81	-	7.53	1.51	5.12	1.2	-	-	0	1.91	-	-
47	58.14	24.28	3.2	1	6.69	1.5	3.5	0.9	-	-	0	0.8	-	-
48	52.71	24.05	3.51	-	9.72	1	5.31	1.1	-	1.1	0.2	1.3	-	-
49	54	26.9	3.3	0.6	7.5	1	2.9	1.3	-	1.1	0.1	1.3	-	-

Table S2 identified protein and specific peptides in the fragment sample.

Protein	Accession number	Taxa	Tissue	Number of identified peptides (unique)	Number of peptide spectral matches	Identified Peptide	Modifications	Score	BLAST taxonomic hit
alpha-gliadin precursor	CAI35909.1	Triticeae	seed	3(3)	6	LPQFEEIR		31.25	Triticeae
						YSQPQQPISQQ		23.33	Triticeae
						QLPQFEEIR		22.92	Triticeae
gamma-gliadin	ADZ76045.1	Triticeae	seed	3(1)	4	GIIQPQQPAQLE VIR		38.3	Triticeae
						QPQQPFPQTQQPQQ PFPQ		17.49	Triticeae
						APFASIVA		16.32	Triticeae
gamma-gliadin	ATD83927.1	<i>Triticum/Aegilops spp.</i>	seed	2(1)	3	GLGIIQPQQPAQLE GIR		22.53	<i>Triticum/Aegilops spp.</i>
						SLVSIILPR		19	<i>Thinopyrum/Triticum/Aegilops/Citrus spp.</i>
gamma-gliadin	ATD83976.1	<i>Thinopyrum/Triticum/Aegilops spp.</i>	seed	2(1)	2	GIIQPQQPAQLEAIR		35.44	Triticeae
						SLVSIILPR		19	<i>Thinopyrum/Triticum/Aegilops/Citrus spp.</i>

putative alpha- amylase inhibitor CM2, partial	CBA13559.1	<i>Triticum aestivum</i>	seed	1(1)	1	GSPVSTEPGNTPR		31.24	<i>Triticum aestivum</i>
Alpha- amylase/tryp sin inhibitor CM3	P17314.1	<i>Triticum spp.</i>	seed	4(4)	9	SGNVGESGLIDLPG CPR	Carbamido methylation	50.54	<i>Triticum/Aegilops spp.</i>
						ALPVPSQPVDPR		28.78	<i>Triticum/Aegilops spp.</i>
						EMQWDFVR	Oxidation (M)	25.87	<i>Triticum spp.</i>
						LPVPSQPVDPR		20.87	<i>Triticum/Aegilops spp.</i>
predicted protein	BAK02811.1	<i>Hordeum vulgare subsp. vulgare</i>	riboso me	1(1)	1	RIEDVTPTPSDTSR		25.11	<i>Hordeum vulgare subsp. vulgare</i>
beta casein	AAA30431.1	Bovinae	milk	8(8)	35	AVPYPQR		28.15	Moschidae/Cervidae/Bovinae
						AVPYPQR	Deamidation (NQ)	17.82	Moschidae/Cervidae/Bovinae
						DMPIQAF	Oxidation (M)	22.12	Cervidae/Bovinae/Caprinae
						LLYQEPVLGPVR		41.34	Bovinae/Caprinae

						LYQEPVLPVVR		34.83	Bovinae/Caprinae
						LYQEPVLPVVR	Deamidation (NQ)	22.51	Bovinae/Caprinae
						YQEPVLPVVR		35.14	Bovinae/Caprinae
						QEPVLPVVR		25.42	Bovinae/Caprinae
						EPVLPVVR		38.84	Bovinae/Caprinae
						GPFPIIV		24.89	Cervidae/Bovinae
alpha S1 casein, partial	ABW98949.1	<i>Muntiacus/Bovinae</i>	milk	2(2)	5	GLPQEVLNENLLR		32.3	<i>Muntiacus/Bovinae</i>
						GLPQEVLNENLLR	Deamidation (NQ)	16.96	<i>Muntiacus/Bovinae</i>
						LPQEVLNENLLR		22.95	<i>Muntiacus/Bovinae</i>