

Supplementary information (SI)

Microbial degradation mechanism of historical silk revealed by proteomics and metabolomics

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Data quality control in proteomics

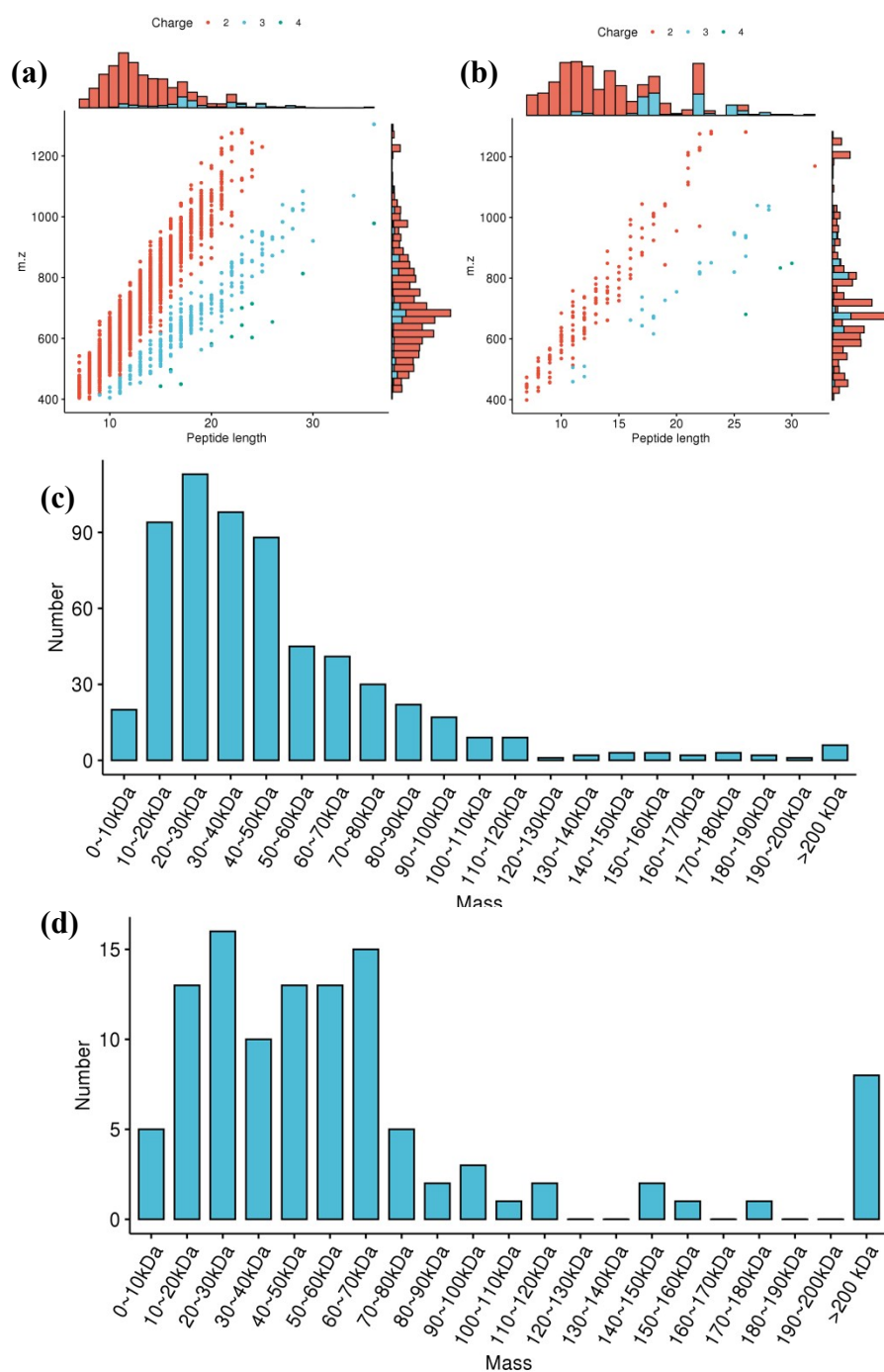


Fig. S1. Peptide length distribution of (a) artificially aged silk samples and (b) historical silk samples. Protein molecular weight distribution of (c) artificially aged silk samples and (d) historical silk samples.

Most of the peptide segments are distributed in 7-20 amino acids, which conforms to the general rule based on enzymatic hydrolysis and mass spectrometry fragmentation, suggesting that the length distribution of peptide identified by mass

spectrometry meets the quality control requirements. In addition, the identified proteins are distributed in all molecular weights.

Data quality control in metabolomics

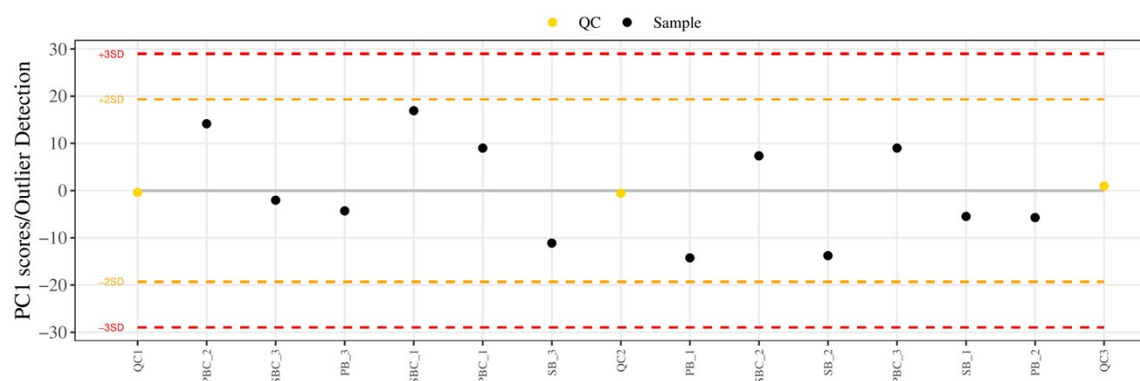


Fig. S2. Multivariate control chart, i.e., the position and changing trend of sample scoring points formed according to sample data of SBC vs. SB and PBC vs. PB.

As shown in Fig. S2, each point represents a sample. The black point is the test sample, and the color point is the QC sample. The X-axis is the machine detection sequence of all samples, and the vertical axis is the first principal component score of the sample principal component analysis. Generally, most points will fluctuate up and down around the X-axis within 2 standard deviations, and points exceeding 3 standard deviations are considered outliers. Apparently, the samples measured in this work are in a controlled state.

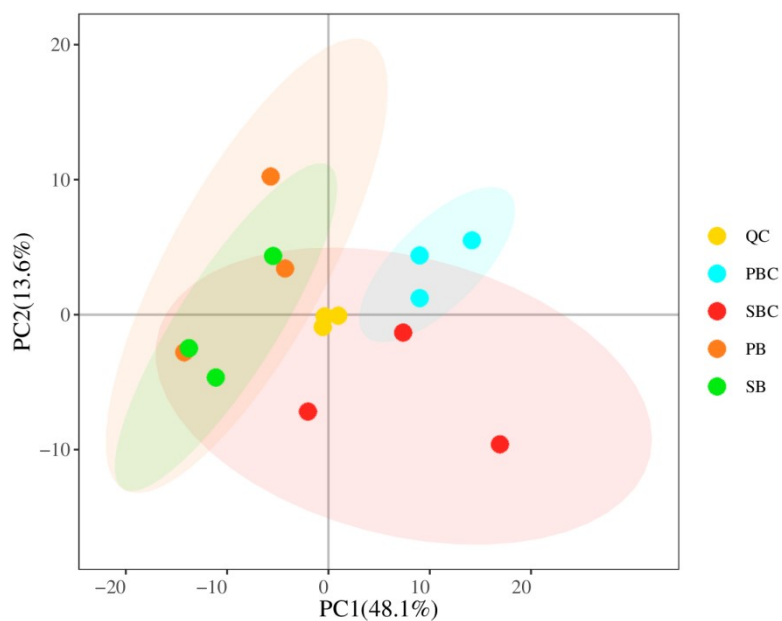


Fig. S3. Principal Component Analysis of all samples (including QC samples).

PCA analysis is an unsupervised method, which helps to observe the original features of the data. In Fig. S3, QC sample points are close to each other and clustered together to a high degree, indicating good stability of instrument detection.

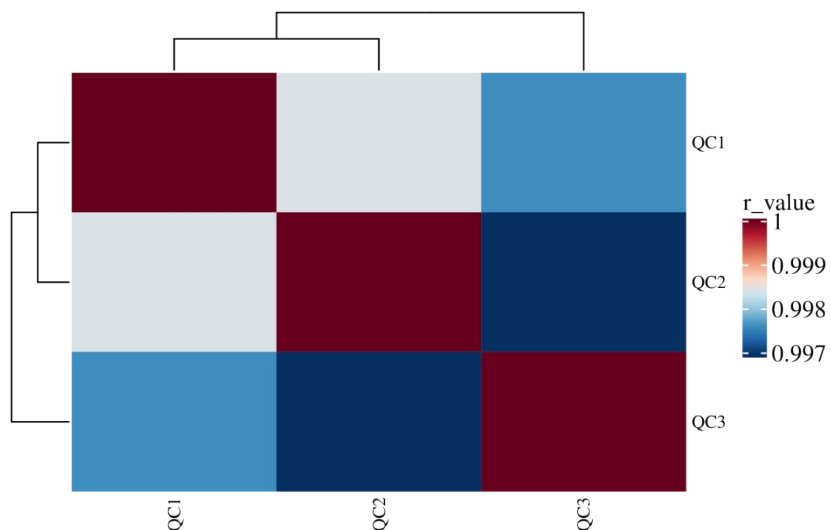


Fig. S4. Correlation heat map among QC samples.

The color of each grid in the heat map represents the correlation coefficient between one QC sample and other QC samples. The closer the correlation coefficient is to 1, the closer the QC samples are, which indicates that the more stable the test data is and the better the experimental quality control effect is.

Morphology of ancient silk fibers

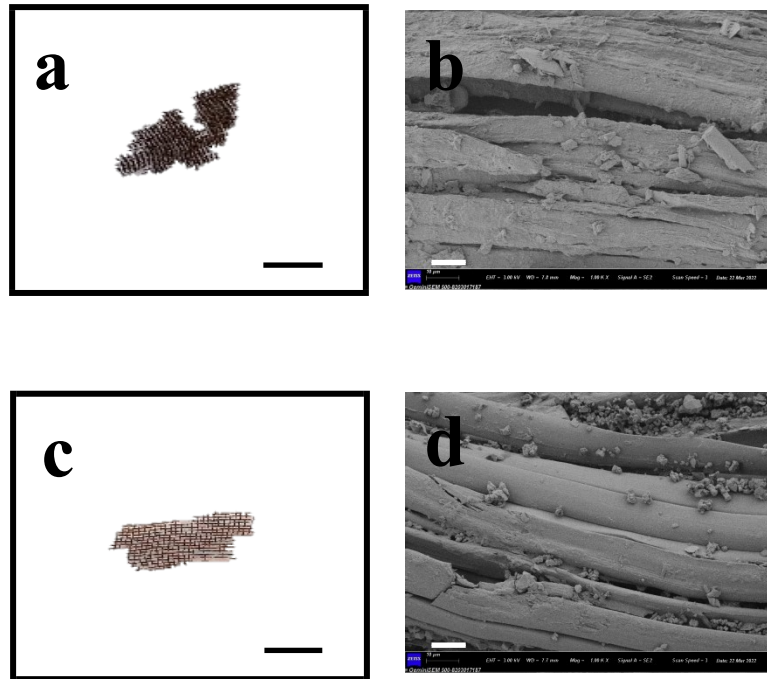


Fig. S5. (a) and (c) are digital images of AS_SX and AS_XJ respectively. Scale bars of digital images are 1 cm. (b) and (d) are SEM images of ancient samples AS_SX and AS_XJ respectively. Scale bars of SEM images are 10 μm .

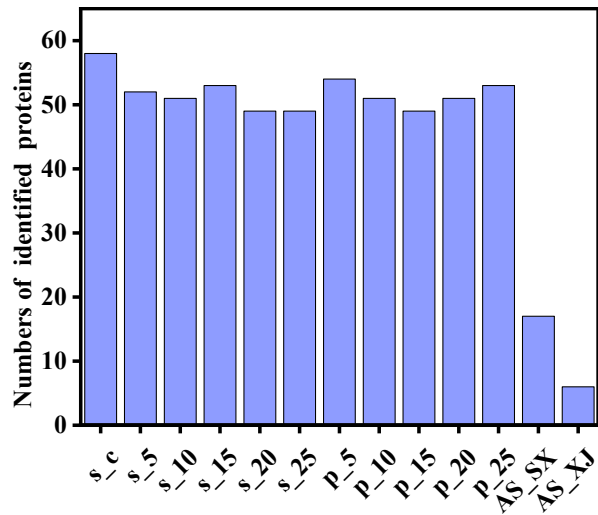


Fig. S6. Numbers of identified proteins in artificially aged silks and historical silks.

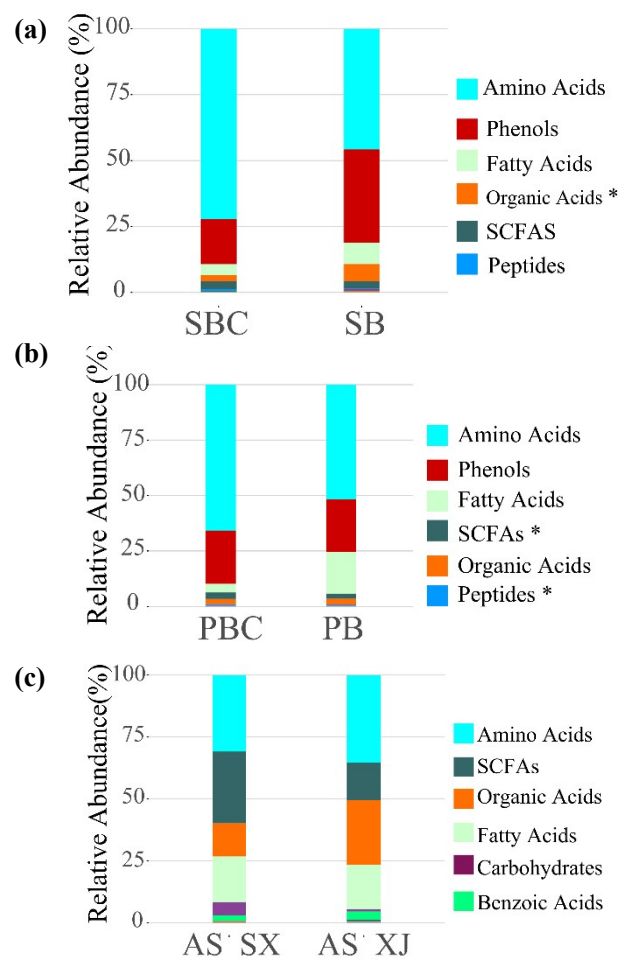


Fig. S7. Relative abundance of different metabolite classes from (a) SBC vs. SB, (b) PBC vs. PB, and (c) archaeological silks. *: classes of metabolites with significant difference ($p < 0.05$).

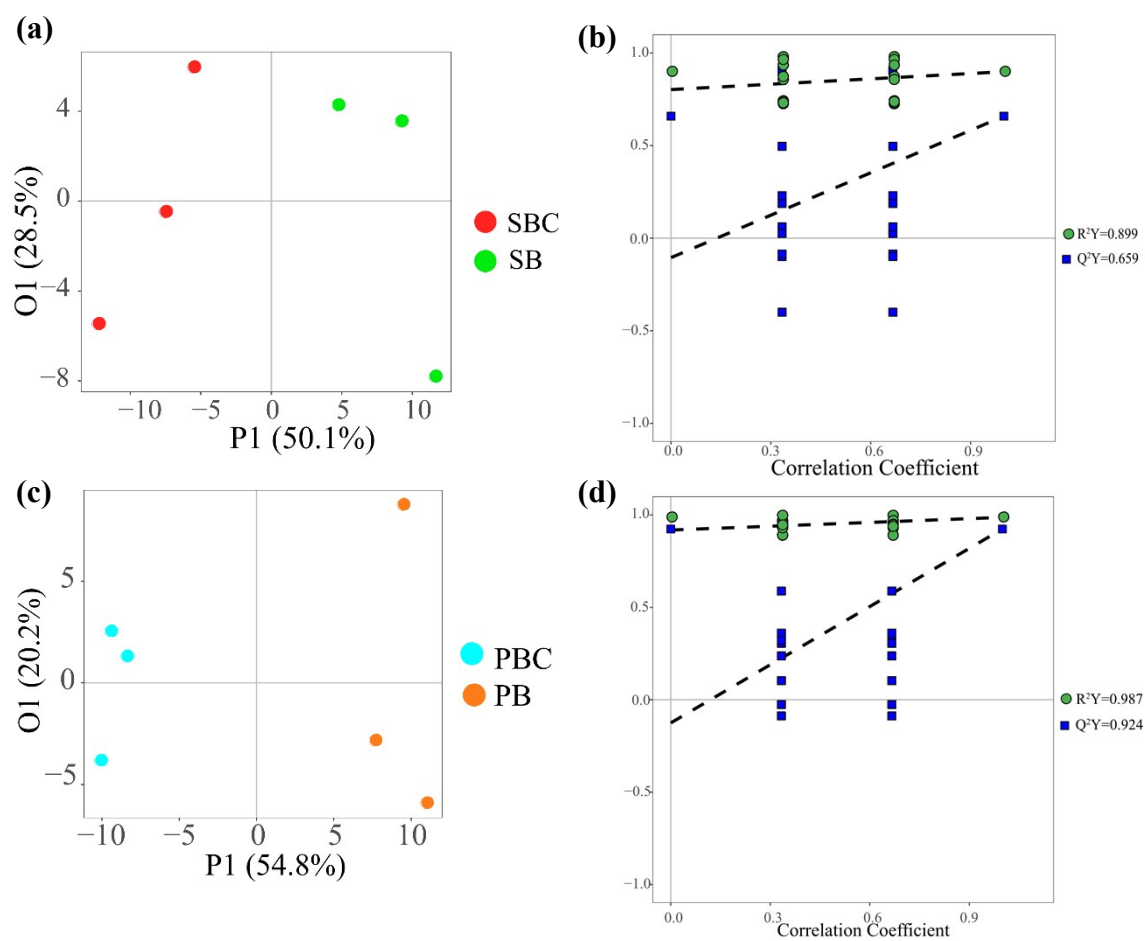


Fig. S8. (a) OPLS-DA model of metabolites from SBC and SB. (b) Permutation test results of the OPLS-DA model of metabolites from SBC and SB. (c) OPLS-DA model of metabolites from PBC and PB. (d) Permutation test results of the OPLS-DA model of metabolites from PBC and PB.

/895623.8653.8528520

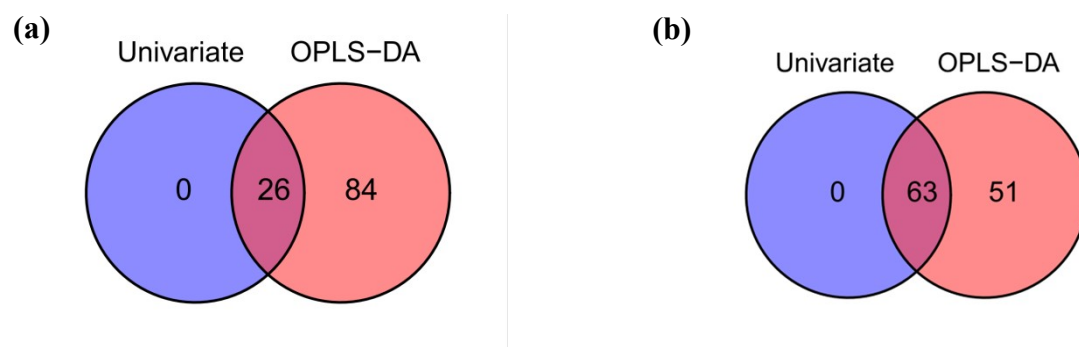


Fig. S9. Screening of differential metabolites. (a) and (b) are Venn plots for the common differential metabolites screened with univariate analysis and multidimensional analysis based on OPLS-DA from SBC vs. SB and PBC vs. PB, respectively.

FTIR and secondary structure

A Tensor 27 infrared spectrometer (Bruker Corporation, Germany) was used to analyze the infrared absorption spectrum of the collected silk samples with a wavenumber range of 400-4000 cm^{-1} . The change in secondary structure was obtained by peak fitting of the amide I region¹ (1600-1700 cm^{-1}). Fourier deconvolution was applied to locate the positions of the peaks. Spectra were deconvoluted with a Gaussian curve in the following steps: (1) The peak position was fixed; the peak width was constrained to a maximum of 50 and a minimum of 5, and the peak area was changed from 0 to 20 limit, and the tolerance was set to 1E-5. After that, iterative fitting was carried out until no change was observed between two consecutive reports. (2) The peak area was fixed, and the fitting was iterated until convergence was achieved.

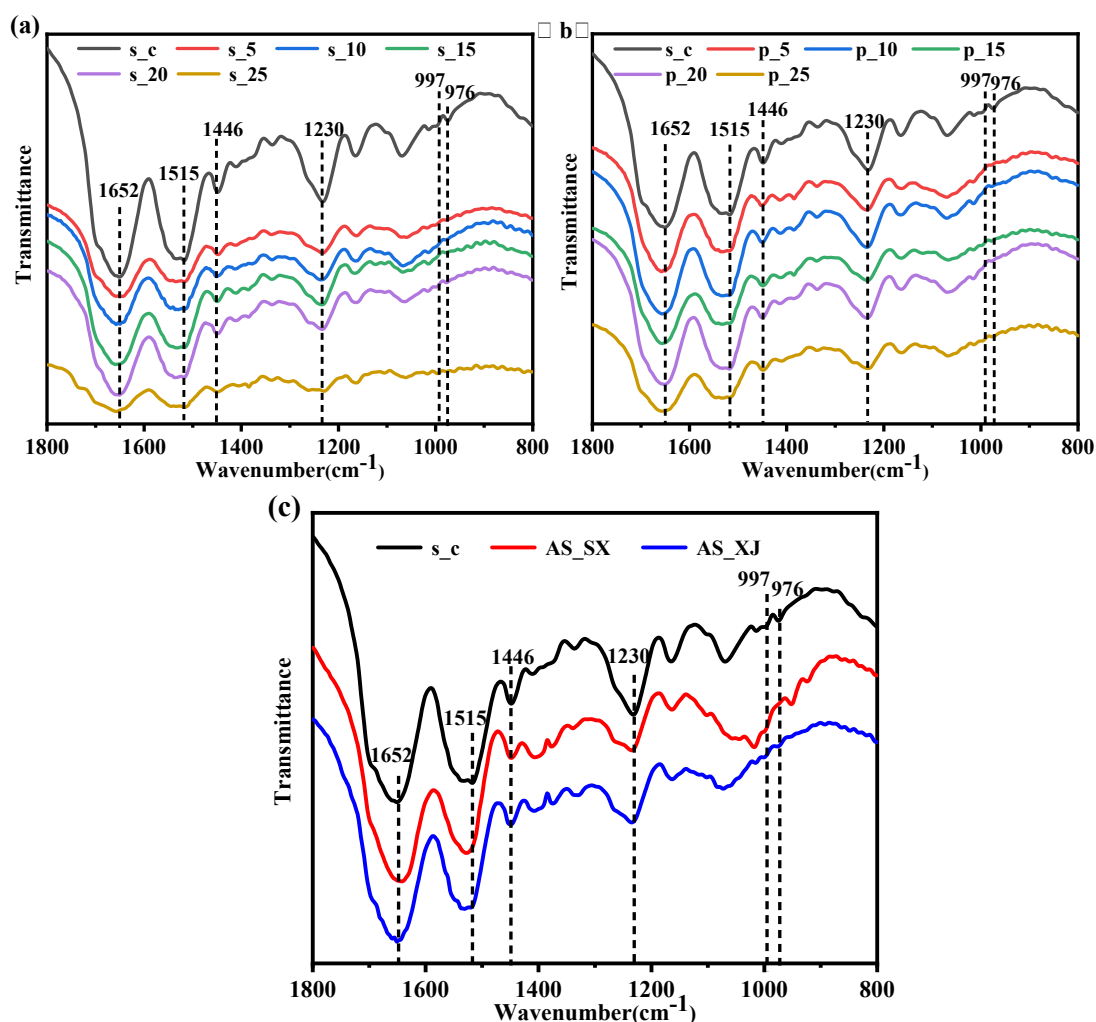


Fig. S10. FTIR spectra of silk samples: (a) and (b) are artificial aging silks treated with *S. maltophilia* and *P. alcaligenes*, respectively; (c) historical silks

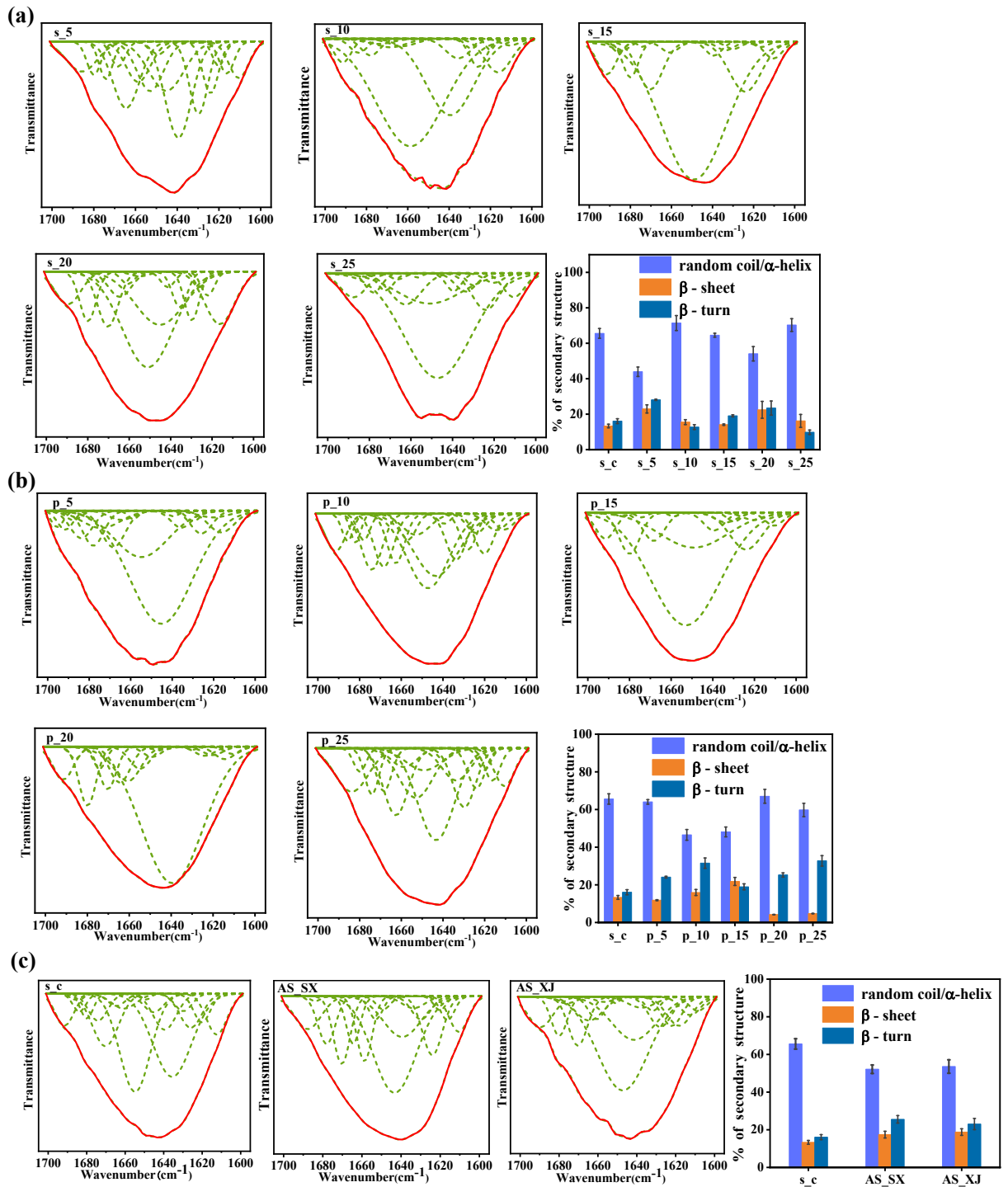


Fig. S11. The deconvolution results of FTIR spectra and the corresponding content of secondary structure of (a) artificially aged silks treated with *S. maltophilia*, (b) artificially aged silks treated with *P. alcaligenes* and (c) historical silks.

The FTIR spectra of all artificial silk samples are shown in Fig. S3. The characteristic absorption peak at 1652 cm^{-1} and 1230 cm^{-1} due to the random coil conformation in the amide I region and amide III region², respectively, and the characteristic absorption peak at 1515 cm^{-1} and 1446 cm^{-1} in the amide II region due

to the β -sheet conformation are observed. After bacterial degradation, the random coil and β -sheet conformations of the silk samples are destroyed. In addition, the characteristic absorption peaks^{3, 4} at 997 cm^{-1} and 976 cm^{-1} from CH_3 rocking of the typical sequence Gly-Ala and C-C skeletal stretching modes of the typical sequence Gly-Gly in *B. mori* silk gradually disappeared, revealing that severe degradation in the silk molecular chain and microcrystalline region composed of regular repetitive sequences occurred.

To comprehensively show the influence of bacterial degradation on the conformation of silk fibers, the change in secondary structure was obtained from deconvolution of the FTIR spectrum (Fig. S4). In the s_5, s_10, s_15, s_20, and s_25 samples, it is notable that the decrease and increase in random coil/ α -helix proportion are accompanied by the increase and decrease in β -sheet and β -turn proportions, respectively. In the p_5, p_10, and p_15 samples, the increase and decrease in random coil/ α -helix proportions are likewise accompanied by the decrease and increase in β -sheet and β -turn proportions, respectively; however, in p_20, the content of random coil increases, and that of β -sheet decreases sharply until the 25th day, and only the β -turn content responds to the change in random coil content. In the antique silk samples AS_SX and AS_XJ, the contents of various conformations are similar, which may be due to the same state of preservation. According to previous work⁵⁻⁸, the changes in secondary structure were attributed to (1) conformations of artificially aged samples are transformed into each other by bacterial biological interactions; that is, β -sheets, α -helices, β -turns, and random coils could be transformed into each other; (2) the collected silk samples are independent of each other, and the changes shown are the consequences of degradation of the silk samples by bacteria, which may result from indirect conformational damage caused by the destruction of the structural sequence and direct conformation damage.

Table S1 Detailed information on historical fabrics.

Sample	Age	Site location	Burial condition
AS_SX	Ming Dynasty (~1504 AD)	Taiyuan, Shanxi Province, China	Unearthed from Ming tomb, hot and rainy in summer, cold and dry in winter
AS_XJ	Han-Jin period (202 BC–420 AD)	Yingpan burial site, Korla City, Xinjiang, China	Unearthed from the ancient castle in the desert, arid and rainless

Table S2 UPLC-MS/MS instrument settings in metabolites quantification.

UPLC	
Column	ACQUITY UPLC BEH C18 1.7 μ M VanGuard pre-column (2.1 \times 5 mm) and ACQUITY UPLC BEH C18 1.7 μ M analytical column (2.1 \times 100 mm)
Column Temp. ($^{\circ}$C)	40
Sample Manager Temp. ($^{\circ}$C)	10
Mobile Phases	A=water with 0.1% formic acid; and B=acetonitrile / IPA (70:30)
Gradient Conditions	0-1 min (5% B), 1-11min (5-78% B), 11-13.5 min (78-95% B), 13.5-14 min (95-100% B), 14-16 min (100% B), 16-16.1 min (100-5% B), 16. 1-18 min (5% B)
Flow Rate (mL/min)	0.4
Injection Vol. (μl)	5.0
MASS SPECTROMETER	
Capillary (Kv)	1.5 (ESI+), 2.0 (ESI-)
Source Temp ($^{\circ}$C)	150
Desolvation Temp ($^{\circ}$C)	500
Desolvation Gas Flow (L/Hr)	1000

Table S3 The identified results of protease inhibitors in artificially aged silk samples treated by *Stenotrophomonas maltophilia*.

Protein accession	Protein description	Intensity					
		s_c	s_5	s_10	s_15	s_20	s_25
P81902	Trypsin inhibitor	107580	0	0	45092	42834	50382
H9IH32	SERPIN domain-containing protein	3662.8	4669.1	0	4114.1	0	0
H9JH31	SERPIN domain-containing protein	396.27	0	0	404.68	0	664.67
P80034	ntichymotrypsin-2	1599.9	1303.9	1259.7	982.75	0	0
Q6Q2D6	Serpin-4A	20451	0	0	0	0	0

Table S4 The identified results of protease inhibitors in artificially aged silk samples treated by *Pseudomonas alcaligenes*.

Protein accession	Protein description	Intensity					
		s_c	p_5	p_10	p_15	p_20	p_25
P81902	Trypsin inhibitor	39281	71929	0	0	47030	62048
H9IH32	SERPIN domain-containing protein	3662.8	5610.1	5211.9	3440.3	4216.6	2936.1
H9JH31	SERPIN domain-containing protein	123.29	295.34	320.9	0	212.99	263.5
P80034	Antichymotrypsin-2	1599.9	2541.5	2272.8	1426.4	1625.2	390.62
Q6Q2D6	Serpin-4A	51717	0	0	0	0	0

Table S5 Quantitative results of metabolites from SBC and SB.

Metabolites (nmol/g)	SBC.Mean	SBC.SD	SBC.Median	SB.Mean	SB.SD	SB.Median
2,3- Diaminopropionic acid	44.7174	10.2107	39.3790	17.5068	4.2329	15.2926
5-Hydroxylysine	2082.5134	368.4747	2016.5732	655.7800	325.80 97	677.5262
Lysine	2756.4441	923.2002	2942.1500	1488.9519	778.14 70	1381.2051
Histidine	1394.5483	493.3550	1525.6831	748.0042	363.11 98	609.8936
Arginine	2295.9378	1097.989 0	2015.1171	2179.9464	998.08 37	1761.8544
Ornithine	62233.7930	24828.18 40	52041.6610	15900.7664	7274.6 155	20092.8718
Glutamine	2009.1278	1109.221 1	1994.5151	948.4902	250.25 76	864.7132
Glutamic acid	6310.7495	1749.727 8	6644.2198	3578.6296	643.74 92	3290.0210
Anserine	34.1588	11.0182	32.2205	11.1438	3.7645	10.3998
Carnosine	224.0116	105.5393	211.3182	55.2329	20.493 2	50.8398
Sarcosine	1184.3924	588.7793	1152.9086	203.7064	80.950 5	198.7593
beta-Alanine	1796.9314	701.2569	1860.7262	517.3631	199.84 93	516.5003
Alanine	72920.0534	11624.37 30	77664.7060	33591.3802	7675.9 494	30953.9378
Dimethylglycine	22.2860	11.8777	19.9315	4.7438	1.6091	4.4739
GABA	909.7896	209.1986	1023.5038	335.1608	122.56 57	321.0583

Serine	8589.6195	2989.478 0	8589.6057	3204.0619	900.59 93	3344.0685
Threonine	4334.6170	1748.684 9	4043.4642	3240.0642	2230.4 064	2424.5312
Homoserine	1016.2442	332.0701	1164.9264	398.8845	180.74 63	305.8763
Creatine	1728.6011	552.5018	1842.5898	517.0288	186.44 46	528.8650
Threonic acid	4.6635	0.5947	4.7867	1.1729	1.2383	0.9050
Hydroxypropioni acid	812.5482	57.4027	780.6831	739.4430	205.36 76	694.6366
Aminocaproic acid	194.5588	110.5917	194.6467	26.6219	17.687 6	20.2899
Glycylproline	5981.9388	3344.599 3	5837.4338	987.2617	487.67 55	903.9106
Homocitrulline	1410.0430	286.7255	1552.2824	523.8345	163.91 03	560.2202
AMP	7.9231	5.3085	10.4186	25.9357	11.489 8	20.8148
GMP	25.0263	11.1730	27.9350	54.7559	66.991 5	27.8315
Lactic acid	609.8674	212.4672	580.3850	277.7379	182.07 76	203.4929
Nicotinic acid	399.5107	153.4004	435.4463	321.6440	34.601 5	324.0805
Methylcysteine	75.5132	26.6378	86.0076	23.0846	22.923 7	11.1821
Gallic acid	37.6800	19.4303	39.5702	4.5416	5.2381	3.2412
Tyrosine	11544.2156	4186.512 9	10638.2866	4113.2044	1208.3 119	4680.1585

Asparagine	2276.5472	1066.831 4	1905.4562	578.9532	164.43 80	648.1269
Protocatechuic acid	14.0023	1.4005	14.5336	11.3263	2.5936	10.2034
Phenylalanine	8237.2874	1327.922 6	8356.3211	4752.6969	1221.1 195	5002.5584
Hydroxyphenyllactic acid	35.1132	4.7325	35.5743	19.2341	3.6054	21.1810
Glycyleucine	1171.0241	738.8853	919.2617	195.3399	67.545 5	206.9621
3-Hydroxyhippuric acid	11.3517	3.1336	11.3680	5.2539	1.1224	4.6145
4-Hydroxyhippuric acid	123.1771	99.8359	90.1042	22.5823	15.145 5	22.8733
Kynurenine	68.8430	36.7246	48.2614	103.3860	48.614 6	118.6663
3-Nitrotyrosine	17.7607	4.5604	19.5373	9.1169	4.8017	9.3303
Aspartic acid	13780.0109	3895.709 3	13322.8898	7414.9440	2026.0 115	6783.5262
Aminoadipic acid	187.2024	16.5411	188.3912	115.8899	37.344 1	126.4754
Glucaric acid	138.3316	42.4053	159.5280	47.4131	14.620 8	40.8276
2-Butenoic acid	433.1761	66.7436	465.3782	244.4979	98.581 4	201.0053
Pyrrole-2-carboxylic acid	48.6810	22.0990	42.2525	18.1562	9.7051	17.6648
2-Hydroxy-3-methylbutyric acid	144.8804	39.0004	145.5678	43.4398	19.179 1	53.2089
4-Hydroxybenzoic acid	195.1559	52.7548	192.9344	87.7522	23.804 4	89.1428

Hippuric acid	3.0577	2.7193	3.1656	0.0101	0.0000	0.0101
N-Acetyalanine	1271.4174	669.0737	1279.1215	327.3804	205.13 22	252.3425
Malic acid	224.3754	46.4096	219.6020	120.5770	56.837 3	145.5641
N-Acetyaspartic acid	937.9805	618.1603	856.9620	263.6100	88.985 1	248.1811
N-Acetytyrosine	44.1100	16.4179	52.0817	16.2016	15.532 2	7.2465
Ethylmethylacetic acid	4200.6775	831.4832	4349.4807	2474.1859	518.21 70	2701.2019
2-Methy-4-pentenoic acid	64.2827	14.0136	57.5933	27.4365	11.342 6	25.8630
Benzoic acid	5.8992	1.5294	6.1815	4.2862	7.1499	0.2889
2-Hydroxycaproic acid	1.1052	0.8802	1.4585	0.9446	1.1044	0.6600
Phenylacetic acid	403.4469	48.9332	429.2161	361.2838	44.658 8	338.3095
Indole-3-carboxylic acid	2.0508	1.1784	1.4599	2.6638	0.4526	2.8365
4-Hydroxycinnamic acid	161.4275	4.2843	160.7168	132.2154	87.648 0	89.3690
Indoleacetic acid	0.4955	0.1038	0.5153	0.3780	0.1692	0.3075
Salicyluric acid	51.3783	9.8706	52.6146	24.7134	8.9476	27.0439
Fumaric acid	57.7820	11.4095	57.2236	67.8220	17.466 1	63.7050
Glutaric acid	25.5394	2.0083	25.0749	33.2102	10.816 5	39.3901

Aconitic acid	103.0389	20.3327	106.2247	118.7047	94.833 9	75.6011
N-Acetyltryptophan	9.4482	1.8214	10.3843	3.3771	2.0853	2.3293
Cinnamic acid	2.2113	1.0413	2.2457	0.9298	0.9419	0.5623
N-Phenylacetylphenylalanine	4.3632	1.2123	4.5274	2.5122	2.1417	1.3293
4-Hydroxyphenylpyruvic acid	194320.9894	205873.2 667	82575.2925	273031.892 5	177957 .8177	183765.3919
Azelaic acid	17.6313	4.1183	19.0924	7.7497	2.5630	8.6725
alpha-Ketoisovaleric acid	59.1992	74.0140	22.5638	107.5611	48.963 7	104.5143
Ketoleucine	1697.6400	1571.056 4	919.1456	3554.8710	1018.5 678	3535.6617
3-Methyl-2-oxopentanoic acid	1246.5167	1394.228 1	543.1984	2376.9116	1208.2 890	2272.5101
Phenylpyruvic acid	1112.6099	1132.058 3	487.8323	2160.5910	1368.4 768	1714.6904
Methylmalonic acid	178.4083	117.6896	234.2502	90.4611	28.097 9	100.8357
2-Hydroxyglutaric acid	94.2845	65.1531	121.1529	16.2123	13.722 3	8.4848
Glycine	316750.6705	98419.51 03	284908.3365	163717.397 9	39096. 6421	178850.6612
Guanidoacetic acid	233.6394	61.1913	227.6749	89.2939	35.431 6	97.6253
Citrulline	43950.9253	8605.395 5	47538.5615	16302.2364	8065.6 529	13826.2234
Galactonic acid	223.4668	71.1602	215.7151	75.2328	31.204 4	79.3641

Gluconolactone	102.6204	24.8697	110.6949	58.9802	5.0262	57.2103
Glycolic acid	363.6228	37.6860	378.7124	235.6665	62.302 9	229.5421
alpha-Aminobutyric acid	12729.8434	6166.108 8	9170.4774	4419.9554	1458.3 333	5215.5064
Glyceric acid	95.2337	20.5707	101.0298	109.9259	50.443 0	100.5913
Proline	93276.7721	32957.05 50	97252.4363	31662.6081	7945.5 606	27199.4650
Acetylglycine	3174.7697	2603.492 8	2052.1256	576.2677	310.01 82	541.5264
Pipecolic acid	14.5945	1.6734	15.5311	8.4665	3.5452	7.8191
Erythronic acid	16.1753	5.2782	14.5218	6.7040	2.3992	6.9150
N-Acetylserine	132.4346	79.0095	87.2659	41.8143	23.177 8	35.4483
Shikimic acid	2124.9655	575.9195	2001.7327	1073.4636	421.84 04	1022.9692
N-Acetylglutamine	9.9892	2.8100	11.0409	7.7723	1.4067	7.5163
Quinic acid	2.9785	0.7089	2.6496	0.4094	0.4593	0.2809
Acetic acid	6550.7725	1658.812 8	6129.2802	4418.2455	2098.2 885	3315.0410
Valine	28502.2833	14640.89 68	20217.5903	8882.4590	3385.7 392	9390.3393
Pyroglutamic acid	19167.4752	10875.18 17	13756.8429	6439.5870	2361.5 526	7627.0547
5-Aminolevulinic acid	1113.3293	816.5430	721.8582	259.3689	107.72 80	285.7057

Glucose	178.7033	107.0575	200.6856	671.2170	902.16 09	299.5260
3,4-Dihydroxymandelic acid	2.5880	0.9853	3.1343	1.4800	0.7440	1.5406
Lactulose	10.2962	4.3427	9.2572	6.0810	1.0160	5.8909
Maltose/Lactose	22.9166	16.5943	24.0185	23.3868	16.425 7	21.6158
Maltotriose	3.8630	1.5542	3.1968	9.7425	10.664 2	4.1164
3-Hydroxybutyric acid	642.6851	211.6522	555.8517	90.8338	19.715 5	85.7466
Methionine	16884.0153	4898.109 3	16942.7366	7492.0618	2034.2 883	7709.6521
4-Aminohippuric acid	26.7377	7.1754	26.9156	11.4788	4.6800	9.1963
2-Hydroxybutyric acid	201.1055	45.0491	225.8521	84.0270	36.521 9	71.6934
3-Hydroxyisovaleric acid	78.5232	25.9898	75.4823	53.0821	3.3467	54.7454
Glutaconic acid	127.5151	62.2992	105.1261	48.3350	11.848 7	41.7112
Isoleucine	15211.7164	6260.092 6	12916.9671	5077.3245	1556.0 955	4181.1161
Leucine	27516.6091	11859.77 43	22871.5588	9905.2808	2532.7 633	8685.9032
Xylose	1262.1024	404.3926	1165.9963	3622.8342	1865.2 026	4183.2653
Ribulose	151.4726	53.3911	121.8932	105.2727	64.423 8	88.2052
Xylulose	104.1073	36.9626	87.4695	81.0090	51.946 1	67.4459

Rhamnose	7.8412	0.9667	7.8952	24.6299	38.975 1	2.2558
beta-D-Fucose	86.5725	39.1493	73.5454	36.2609	7.4191	33.2771
p-Hydroxymandelic acid	8.5486	1.1285	8.8812	6.5324	5.3920	3.8075
Fructose	5.8221	0.8879	5.8399	2.4622	1.0967	1.8748
N-Acetyl-D-glucosamine	67.0186	44.4629	45.9709	84.4499	30.280 3	97.7697
Propionic acid	10233.7165	2922.896 3	9955.0522	6150.0578	2569.9 654	5132.4003
Glyceraldehyde	50.3986	59.9093	21.3736	56.5044	12.587 9	51.5444
Norleucine	78.8658	49.7306	62.9559	13.3035	4.5774	15.8490
Homovanillic acid	21.3071	5.8811	22.8321	10.8602	3.3248	11.4853
2-Furoic acid	41.7426	7.9181	46.2782	23.2580	7.6655	26.4940
p-Hydroxyphenylacetic acid	26.8859	4.9876	24.6665	29.2299	11.778 7	23.4110
Tryptophan	1406.3315	403.669	1631.7428	648.9399	229.19	529.8226
Butyric acid	230.7566	54.1338	256.9489	180.1910	68.649 5	155.9040
Isobutyric acid	6069.4670	1373.623 4	6167.8363	4238.5145	1085.0 682	4500.7958
Malonic acid	251.1170	78.2219	281.6208	84.8458	30.090 0	85.3085
Tartaric acid	1.0106	0.2563	0.9596	0.8570	0.1289	0.7969

Isovaleric acid	4958.5304	1221.179 7	5000.7309	2905.0195	484.69 57	3128.2158
Valeric acid	37.7597	7.1135	36.3913	24.3874	3.9176	22.8297
N- Methylnicotinamide	808.6118	237.8849	858.6994	559.9137	32.656 9	574.1470
Phenyllactic acid	227.6778	81.3387	205.8026	112.7845	22.443 2	120.3587
Oxalic acid	949.3080	322.1144	1027.4939	315.3309	136.50 34	287.0454
Maleic acid	33.9924	16.6011	25.4287	10.7339	0.4499	10.8193
Itaconic acid	6.7808	2.9000	8.1013	2.7110	1.1386	2.3983
Methylsuccinic acid	28.7697	8.0823	31.4680	18.7274	3.9940	19.0632
Adipic acid	8.5346	1.6449	8.9646	4.3328	2.9798	5.1890
Phthalic acid	3.0503	0.8958	3.1537	0.6736	0.6275	0.6972
Pimelic acid	5.2619	0.4313	5.1084	3.1084	1.4038	3.9060
3-Methylpentanoic acid	330.3148	65.8813	309.8093	386.7637	39.100 6	365.6329
Isocaproic acid	24.1300	3.2307	23.5535	22.0009	2.3749	21.7726
Caproic acid	11.1175	1.3937	10.9770	14.0377	6.8489	12.5970
Acetoacetic acid	3897.9064	519.7653	3825.5922	26695.1198	7123.1 779	29397.7735
Suberic acid	6.3883	2.3293	6.7090	2.9481	1.4462	3.3914

TCA	4.0067	1.0909	4.3783	1.3694	1.1084	1.8105
Citric acid	394.2590	130.2043	462.4932	403.4287	200.26 41	406.5112
Isocitric acid	69.4221	41.9240	68.4358	47.0964	19.033 3	55.7449
2-Methylhexanoic acid	3.5224	0.9627	3.9000	4.6114	1.6938	3.8509
4-Methylhexanoic acid	238.5843	40.8673	252.5885	133.1284	7.3756	133.8793
Heptanoic acid	8.9414	1.3736	9.1706	9.6771	4.1822	7.9043
Pyruvic acid	9236.0500	2907.271 6	10188.5285	8293.2698	5450.8 044	5912.5773
7-DHCA	0.3757	0.1682	0.2997	0.4055	0.2007	0.3669
Oxoglutaric acid	3393.6961	2295.292 0	4298.3003	4520.7552	3167.5 824	4198.3453
Octanoic acid	211.1409	207.4031	110.5482	374.2340	198.87 08	357.0808
HDCA	0.9469	0.4992	0.7447	0.4658	0.2716	0.4087
CA	3.0967	1.1911	3.1103	1.9334	1.0800	1.6709
GCA	4.9626	1.7037	4.9008	1.9279	1.1243	1.4832
Nonanoic acid	6.8206	1.6102	6.4609	7.3742	8.0543	5.7875
CDCA	1.2866	0.2529	1.2936	1.3479	0.3363	1.2829
Undecanoic acid	20.9250	2.6341	20.4240	29.6865	17.949 8	21.2128

Tridecanoic acid	1.8251	1.0045	1.5394	1.7303	0.6292	1.4299
Myristoleic acid	2.7163	0.9382	2.3147	2.6616	1.0122	2.5328
9E-tetradecenoic acid	0.6759	0.3784	0.5216	1.2925	0.3130	1.2062
DCA	2.4020	0.3219	2.3097	3.5914	3.7074	1.8970
Myristic acid	120.5361	28.4170	123.6598	79.7744	34.299 4	97.9475
9-Pentadecenoic acid	187.5210	73.6546	160.9653	783.6602	1030.7 155	195.0930
Pentadecanoic acid	4026.8127	1415.859 2	3224.6880	6652.0306	5805.5 097	3362.5381
Palmitoleic acid	1209.7477	493.0141	1086.2510	3617.3775	4618.7 166	1003.5418
Palmitelaidic acid	202.3578	87.6471	180.8196	541.8257	574.94 20	222.2791
10Z-Heptadecenoic acid	56.9678	23.1099	66.1641	31.7619	14.627 2	35.6416
10-Trans-Heptadecenoic acid	521.0663	121.8311	569.2998	362.5016	139.61 19	362.8776
Succinic acid	1220.0784	420.6128	1115.1773	1019.2801	204.81 54	1074.3884
Citramalic acid	21.5125	8.7991	22.1778	6.6262	2.0943	5.9255
Palmitic acid	29904.7297	11217.24 44	23900.4816	35244.5910	28572. 3824	21269.4118
Heptadecanoic acid	909.3565	345.4606	734.4809	875.3596	583.69 00	590.1418
Oleic acid	4098.5812	1766.586 5	3411.5832	6955.5038	6628.0 059	4125.4999

Petroselinic acid	815.6806	79.4640	783.4228	1441.8956	1326.4 619	701.8258
Stearic acid	3503.3672	908.7091	3200.2490	4138.3090	2072.9 166	3429.4428
10Z-Nonadecenoic acid	41.2564	23.3204	30.1486	42.6312	37.641 6	22.9831
Carnitine	485.4083	134.9897	541.2780	399.0911	216.53 41	285.0136
Malonylcarnitine	0.5618	0.2714	0.4318	0.0658	0.0500	0.0872
Glucose 6-phosphate	45.0123	37.1903	25.4217	26.6148	16.960 2	18.4558
isoDCA	0.0896	0.0042	0.0909	0.0617	0.0462	0.0852
Norvaline	19582.3262	10995.18 06	17777.3605	3874.1733	1546.3 819	4487.4072
SAH	1.4126	0.5887	1.1523	0.6475	0.2171	0.6003
UCA	0.0997	0.0754	0.1349	0.1155	0.0903	0.1493
12-KetoLCA	0.1783	0.0828	0.1809	0.5881	1.0148	0.0023
TDHCA	1.6470	1.8841	1.1984	0.6002	0.7649	0.3039
N-Acetylhistidine	7.2858	5.9074	5.2698	1.1668	0.1465	1.0861
Indolelactic acid	10.0564	1.7267	9.0917	5.0643	1.7979	5.1631
Indole-3-pyruvic acid	12.1309	10.5085	12.9405	135.1505	76.589 7	122.1839
1-Methylhistidine	45.3669	15.4742	44.0916	16.2423	5.5852	17.8131

4-Hydroxyproline	16351.8261	4195.739 6	16937.0895	7127.0743	1698.1 004	6896.0153
gamma- Glutamylalanine	613.0626	57.0355	602.9926	311.1270	51.479 3	328.0270
Imidazolepropionic acid	16.3139	5.8374	17.2508	5.1369	2.4744	5.2113

Table S6 Quantitative results of metabolites from PBC and PB.

Metabolites (nmol/g)	PBC.Mean	PBC.SD	PBC.Median	PB.Mean	PB.SD	PB.Median
2,3- Diaminopropionic acid	44.9810	4.7891	44.8523	15.6833	7.1161	12.1502
5-Hydroxylysine	2887.5267	248.0942	2893.6985	932.6411	162.539 4	945.2218
Lysine	3826.0228	359.3471	3624.3513	2443.7484	945.818 4	2174.3100
Histidine	1440.7794	267.2662	1434.0178	1077.7022	686.147 4	988.5461
Arginine	3769.5877	677.2155	3750.4898	3041.6775	1870.05 33	2547.7085
Ornithine	90254.7029	12936.476 7	83927.1765	44489.8540	21196.9 086	54224.4127
Glutamine	3009.8983	779.0682	3408.9765	2725.3296	2321.84 37	1795.8629
Glutamic acid	6628.4310	1157.3685	6511.9568	3911.7752	940.979 6	4245.9178
Anserine	34.6059	2.2746	35.3203	13.5626	4.5922	14.8162
Carnosine	227.4759	6.6427	230.1294	80.5087	36.1246	97.3760
Sarcosine	1754.7050	373.3300	1769.8819	482.4335	308.882 6	503.0443
beta-Alanine	1852.0950	66.0315	1837.2619	661.5830	378.057 1	730.6800
Alanine	107651.0685	21638.467 1	112398.8309	61280.5857	21032.0 704	65432.1839
Dimethylglycine	21.3311	5.5167	20.0728	6.4195	4.0879	8.4149

GABA	1294.2125	117.5328	1313.9492	565.4251	315.204 6	581.6728
Serine	11792.7660	1262.6772	11193.6465	6684.5107	2797.08 48	7867.2391
Threonine	52383.6522	10886.178 4	55385.6531	21049.0423	16417.0 460	26803.4525
Homoserine	1743.1643	832.8769	1443.1575	816.5106	577.438 4	676.9907
Creatine	2608.2498	325.4701	2772.3592	923.9328	557.674 6	940.9499
Threonic acid	3.6447	0.9029	3.8136	0.5133	0.7323	0.0905
Hydroxypropionic acid	842.9999	14.3248	835.3291	651.6760	67.4573	644.2384
Aminocaproic acid	123.9232	21.2097	118.0807	33.1429	11.0518	36.5822
Glycylproline	6162.0315	1651.8503	5323.4138	1900.9292	869.692 1	2306.3109
Homocitrulline	2609.1417	425.6328	2584.1086	994.5240	643.308 7	962.8221
AMP	20.1667	13.8298	25.4445	12.8542	6.1443	9.7347
GMP	151.1360	181.7746	84.4089	51.1921	65.4206	18.3953
Lactic acid	752.2127	247.7768	620.2339	175.5967	124.375 5	157.1350
Nicotinic acid	384.5311	40.1638	371.2354	180.1703	57.2276	183.7084
Methylcysteine	189.0416	30.0126	198.4711	58.8641	35.3370	75.6002
Gallic acid	11.7134	4.5654	12.9456	0.3046	0.3957	0.0762

Tyrosine	12996.0000	2300.7957	11789.0489	6758.8512	2517.36 98	7940.1267
Asparagine	3009.8072	599.0344	3317.8422	1465.7630	817.957 0	1525.2997
Protocatechuic acid	12.0658	0.4671	12.0725	7.7848	0.5261	7.8268
Phenylalanine	11841.3135	1413.1752	11192.9316	7462.0186	3024.93 70	7986.6810
Hydroxyphenyllactic acid	10.8144	2.1791	10.3887	9.2324	4.1331	7.0534
Glycyleucine	1206.8402	302.3966	1071.0820	482.3184	247.196 3	545.6814
3-Hydroxyhippuric acid	13.1497	2.2499	12.2069	6.6403	2.3918	5.5046
4-Hydroxyhippuric acid	117.9990	22.1527	105.7554	42.0075	10.4199	37.2624
Kynurenine	110.4247	44.4134	114.9595	89.8965	55.4625	61.7989
3-Nitrotyrosine	21.5771	1.7760	21.2545	11.4782	1.9325	10.9372
Aspartic acid	12668.3495	1747.0698	11670.1664	8698.9853	3425.96 12	8414.5821
Aminoadipic acid	231.4329	34.3882	214.8309	128.5800	29.9276	137.6216
Glucaric acid	152.6646	14.9490	152.4390	62.7907	24.5617	57.1869
2-Butenoic acid	480.7889	40.4372	474.6756	253.1597	90.9562	261.5558
Pyrrole-2-carboxylic acid	45.0296	4.6712	43.5133	21.3804	6.1273	24.6080
2-Hydroxy-3-methylbutyric acid	74.3229	15.6254	82.9882	29.5207	12.6207	28.7778

4-Hydroxybenzoic acid	228.6559	28.7761	222.5897	110.7344	46.4105	110.4547
Hippuric acid	3.8252	1.8020	2.9871	0.1569	0.1815	0.1008
N-Acetyalanine	1041.1975	102.5083	1014.2854	422.5658	183.115 0	331.7918
Malic acid	263.0655	40.6931	280.4085	129.2266	73.4846	102.5247
N-Acetyaspartic acid	626.9064	92.8908	627.6441	225.8350	116.022 1	207.6044
N-Acetytyrosine	54.4184	19.8410	55.1315	23.8352	9.6778	22.0925
Ethylmethylacetic acid	6212.1551	513.6063	6112.5060	3105.8519	1276.83 91	2898.6407
2-Methy-4-pentenoic acid	91.2412	27.6616	95.6613	50.3622	26.6038	63.0818
Benzoic acid	7.8080	2.1919	6.9343	5.8825	3.9327	4.6382
2-Hydroxycaproic acid	3.2058	5.5346	0.0103	0.8703	1.4895	0.0103
Phenylacetic acid	469.7482	62.2164	448.9882	343.2888	37.4036	329.2167
Indole-3-carboxylic acid	2.0636	0.5148	2.3390	2.6920	1.4252	2.3580
Coumaric acid/4-Hydroxycinnamic acid	182.7188	37.3244	178.3409	105.7829	5.9326	102.5125
Indoleacetic acid	0.3630	0.0534	0.3783	0.2827	0.0491	0.2907
Salicyluric acid	59.5879	8.4485	55.6732	25.8064	10.2602	27.3410
Fumaric acid	52.2550	10.1641	55.5603	39.9923	12.6322	35.1115

Glutaric acid	31.9347	6.9510	30.4597	21.7000	3.6898	21.7428
Aconitic acid	150.1352	26.2838	163.6984	48.1995	15.9558	41.7692
N-Acetyltryptophan	13.7072	1.8710	12.8850	4.3520	3.5155	3.2314
Cinnamic acid	3.6329	0.7056	3.9034	0.7306	0.1298	0.6629
N-Phenylacetylphenylalanine	5.5231	2.6970	4.5253	3.5143	2.2900	3.7971
4-Hydroxyphenylpyruvic acid	329356.7069	152957.2662	390070.7450	239878.8934	181607.9539	169949.9862
Azelaic acid	12.1008	1.6368	11.5938	8.3090	2.9259	9.8831
alpha-Ketoisovaleric acid	98.5934	48.3514	115.9328	44.6882	40.7992	24.5118
Ketoleucine	2930.6200	1041.8531	3400.3621	2311.1623	1239.3270	1823.1850
3-Methyl-2-oxopentanoic acid	2354.9764	1139.8673	2588.9974	1373.8851	1161.1720	741.5180
Phenylpyruvic acid	1847.1763	840.8529	2282.5571	1625.5197	1064.6516	1088.0110
Methylmalonic acid	68.1936	39.5385	49.5149	111.1375	62.9577	125.1326
2-Hydroxyglutaric acid	56.0644	30.5530	40.4616	49.0390	33.8456	41.9796
Glycine	303880.8327	50057.0403	296572.2045	202409.4361	40373.2942	223871.3750
Guanidoacetic acid	216.4256	52.0634	217.9354	95.4238	33.0107	103.1866
Citrulline	3297.1661	275.7473	3218.1386	4842.3368	5314.0373	2066.2844

Galactonic acid	219.9844	56.0119	239.8608	83.5914	36.8333	86.3040
Gluconolactone	162.7906	3.1930	162.9731	71.2999	42.0776	95.1275
Glycolic acid	369.4625	18.3216	359.9970	248.7339	56.9306	246.5859
alpha-Aminobutyric acid	3510.4910	1221.3358	3999.1300	3197.5741	1618.20 40	2786.6725
Glyceric acid	89.2533	2.9282	90.5296	53.5952	10.9350	54.2178
Proline	98801.6905	16651.044 8	89720.4784	50987.2104	16326.9 632	60361.9987
Acetylglycine	2505.2291	710.0527	2650.0987	954.7136	283.082 4	1077.5250
Pipecolic acid	14.7267	2.0481	13.7634	7.4922	2.4900	7.0211
Erythronic acid	13.7018	1.0396	13.4916	6.7664	1.9338	7.0690
N-Acetylserine	288.2584	85.3958	332.0764	148.6682	67.5187	167.9934
Shikimic acid	2107.9847	57.0249	2131.0887	1037.9823	283.971 6	1103.1239
N-Acetylglutamine	10.3462	2.3432	9.8370	7.3665	1.6361	6.6436
Quinic acid	3.0435	1.3845	3.3971	0.6411	0.2518	0.6867
Acetic acid	7266.8003	1117.7436	6967.2222	5240.0796	1064.69 06	5385.3217
Valine	26669.5982	5620.7137	27425.2625	14943.8997	5395.20 73	15856.4981
Pyroglutamic acid	16389.4560	4707.3136	17888.2881	8059.9108	2941.63 69	8533.8562

5-Aminolevulinic acid	883.9524	248.2277	984.9765	377.7559	106.242 5	400.2012
Glucose	459.2212	50.0155	441.4850	242.1905	154.378 8	306.0527
3,4-Dihydroxymandelic acid	2.8173	0.9231	3.2504	1.1189	0.2287	1.0279
Lactulose	7.9939	0.5654	7.9306	8.9510	4.8943	6.6450
Maltose/Lactose	8.3395	6.5564	5.7497	11.3169	6.2135	8.5680
Maltotriose	1.0652	0.1776	0.9959	3.9044	3.7538	2.0968
3-Hydroxybutyric acid	1508.0222	134.0585	1455.2061	490.7638	265.316 2	559.6367
Methionine	18649.4124	2957.2076	17347.5054	10604.9477	3614.98 66	12607.4175
4-Aminohippuric acid	30.6659	4.1387	32.1588	8.9576	3.1936	10.1018
2-Hydroxybutyric acid	12.5110	3.1062	14.2444	19.2108	11.8862	20.1978
3-Hydroxyisovaleric acid	117.5395	13.6865	111.7605	53.5354	23.5118	58.5292
Glutaconic acid	144.9321	27.0066	145.3841	72.0375	30.5615	72.2397
Isoleucine	16320.5152	2987.5947	15369.0972	10015.3436	4013.12 19	10790.8603
Leucine	27827.5919	4265.0074	26221.3497	17327.5781	6176.05 93	18179.4237
Xylose	733.7068	255.4362	728.7566	1810.4132	2004.39 34	678.1461
Ribulose	97.9933	7.7551	99.8635	69.5282	44.2155	75.6259

Xylulose	73.9499	4.3581	74.5181	52.3127	34.7473	53.7624
Rhamnose	2.7240	0.9219	2.9344	2.2983	1.3530	1.5239
beta-D-Fucose	32.4031	5.0327	30.9320	16.0964	5.2111	16.7297
p-Hydroxymandelic acid	10.5370	1.1202	9.9702	6.4288	2.3144	5.8820
Fructose	5.9587	1.5574	6.5740	3.9058	1.9700	4.4311
N-Acetyl-D-glucosamine	62.2981	22.7241	68.3833	73.1775	22.4821	81.8232
Propionic acid	7649.2378	869.0063	8146.5182	4975.4518	1306.50 39	5414.6560
Glyceraldehyde	58.8024	46.9576	39.3598	26.9217	21.1756	18.9348
Norleucine	82.5292	18.7101	80.5677	35.9098	19.7754	43.2213
Homovanillic acid	6.7178	0.6845	7.0291	4.5689	1.5668	4.5358
2-Furoic acid	49.4321	14.3063	55.0064	27.2786	5.5605	25.7343
p-Hydroxyphenylacetic acid	34.9060	6.7868	35.2155	21.2660	4.0406	20.6977
Tryptophan	1626.0112	52.1906	1596.5090	1031.7407	419.352 5	1091.5164
Butyric acid	371.3146	18.9013	367.2191	187.3363	69.1889	205.2083
Isobutyric acid	9740.6788	192.1841	9756.4038	5144.5707	2034.80 28	5065.7939
Malonic acid	275.7630	1.1899	276.2008	96.1423	47.0636	108.7261

Tartaric acid	1.0293	0.1353	1.0979	0.8547	0.0847	0.9008
Isovaleric acid	7478.2554	591.1430	7376.4645	3781.2215	1516.94 63	3613.2584
Valeric acid	57.5321	5.9829	55.5993	29.6173	3.0848	29.2151
N- Methylnicotinamide	868.9630	248.3913	735.9557	517.9543	102.142 8	490.6925
Phenyllactic acid	16.7011	5.0537	13.9185	31.4439	44.9364	8.9591
Oxalic acid	1012.9648	258.4880	1095.9234	370.2605	155.681 8	452.8468
Maleic acid	26.7164	7.4681	24.6905	10.5973	6.6289	11.6831
Itaconic acid	12.0724	4.2476	13.4805	4.2822	2.7806	4.0726
Methylsuccinic acid	35.1290	1.5943	35.0512	14.2744	5.6125	15.4080
Adipic acid	6.2582	3.2130	6.6093	5.1915	1.6365	5.3817
Phthalic acid	2.7339	0.5671	2.4572	1.2481	1.0075	1.0624
Pimelic acid	5.5070	2.1316	4.5791	2.6438	0.4776	2.5426
3-Methylpentanoic acid	446.9077	30.4746	439.1407	464.8397	45.5950	440.9756
Isocaproic acid	35.8668	2.6542	36.9906	20.3166	7.9182	22.6620
Caproic acid	12.6951	2.7048	13.3728	13.7589	7.5249	15.6698
Acetoacetic acid	4441.1573	1204.9962	4924.3724	7525.3832	5928.68 02	5165.9630

Suberic acid	4.4633	0.8909	4.8549	3.0488	0.7187	2.7181
TCA	5.9558	2.0695	5.1774	2.6902	1.4238	3.1899
Citric acid	464.9040	72.9633	472.7691	197.5419	66.6992	194.8774
Isocitric acid	74.1536	16.5215	68.6128	37.1655	29.7013	27.1728
2-Methylhexanoic acid	4.5959	0.9801	4.4884	6.0627	1.9852	6.7982
4-Methylhexanoic acid	349.7851	29.2846	357.4424	156.8001	77.8919	166.8576
Heptanoic acid	9.2780	2.1921	9.6889	12.1926	5.9445	15.1921
Pyruvic acid	7039.4097	550.6459	7251.7819	8130.0753	5638.68 28	5538.7119
7-DHCA	0.4201	0.0846	0.4403	0.2649	0.0383	0.2796
Oxoglutaric acid	5732.6118	1035.2983	5699.3814	2674.4111	1539.71 22	2441.8461
Octanoic acid	329.6017	150.4503	390.4774	234.1437	146.677 8	196.4680
HDCA	0.6990	0.1450	0.7279	0.1896	0.2373	0.1003
CA	4.4747	0.5447	4.5399	2.3528	0.9767	2.6398
GCA	3.9947	0.6592	3.9394	2.0113	0.6952	2.4016
Nonanoic acid	6.9810	5.0702	4.7267	14.0097	13.0386	12.4703
CDCA	1.3092	0.7000	1.4182	0.9697	0.4520	1.2002

Undecanoic acid	16.3534	0.9463	16.5978	34.0641	40.6035	15.0355
Tridecanoic acid	1.3693	1.0027	1.1974	1.2233	0.5701	0.9534
Myristoleic acid	4.3710	0.8968	4.8047	5.2717	2.0290	5.2989
9E-tetradecenoic acid	1.2605	0.4435	1.2616	1.7776	0.7029	1.5081
DCA	1.1157	0.4685	0.9340	1.8540	0.7845	2.0139
Myristic acid	128.0423	86.2164	79.9281	114.6957	28.8827	123.8086
9-Pentadecenoic acid	221.2080	65.4916	197.7869	1097.8507	715.687 3	1402.1004
Pentadecanoic acid	4051.5980	1311.9857	3989.0959	13257.4205	7340.47 08	16890.4091
Palmitoleic acid	4472.8370	1134.6775	3931.0505	14897.7476	10537.7 980	20436.1042
Palmitelaidic acid	209.1225	66.4982	171.6900	1033.3794	593.491 3	1197.2773
10Z-Heptadecenoic acid	94.0723	88.3639	45.5307	67.0498	23.1390	53.9397
10-Trans-Heptadecenoic acid	629.0932	472.6595	361.8093	521.1831	93.4136	529.7012
Succinic acid	916.9519	61.5123	923.4251	609.1365	235.949 9	473.2386
Citramalic acid	42.9237	3.1688	43.4750	15.9836	9.0264	17.3678
Palmitic acid	31509.7501	15965.013 1	30517.3334	111932.867 2	71036.9 783	137727.2460
Heptadecanoic acid	840.2454	661.6766	565.1385	2557.2518	1908.34 38	2745.0138

Oleic acid	5763.7998	3547.3195	5751.1626	31376.7839	23942.4 884	43880.9221
Petroselinic acid	348.4594	186.9691	273.4281	1883.9514	871.282 1	2228.0459
Stearic acid	3435.9573	3040.6914	2142.5497	9464.2298	6369.89 53	10744.4740
10Z-Nonadecenoic acid	39.9553	47.8699	20.4860	164.0015	133.256 5	212.7254
Carnitine	180.4777	22.0128	172.0778	117.3755	58.7316	90.7263
Malonylcarnitine	0.4527	0.2327	0.3857	0.1332	0.1081	0.1870
Glucose 6-phosphate	35.4024	19.5720	39.9434	8.6857	3.6019	7.6735
isoDCA	0.1200	0.0237	0.1235	0.2685	0.1583	0.3268
Norvaline	19697.5142	6981.9458	18434.7485	7379.0137	3497.04 50	9190.5945
SAH	1.6435	0.8421	1.1610	0.7862	0.1783	0.8846
UCA	0.0960	0.0720	0.1315	0.1131	0.0880	0.1472
12-KetoLCA	0.3504	0.0366	0.3509	0.2200	0.3411	0.0234
TDHCA	3.7721	1.2504	3.8834	0.3701	0.3967	0.2777
N-Acetylhistidine	5.6108	3.8795	3.7555	1.3681	0.4287	1.4817
Indolelactic acid	0.2880	0.0000	0.2880	1.1715	1.5303	0.2880
Indole-3-pyruvic acid	20.9884	12.4140	15.3316	101.4898	91.1497	54.6694

1-Methylhistidine	49.6160	2.9440	49.6993	21.7133	7.8764	25.3164
4-Hydroxyproline	22291.0138	2781.3963	22856.6283	10635.3101	4369.95 61	11275.0459
gamma- Glutamylalanine	717.6423	206.6229	691.5525	437.9356	196.345 9	448.6914
Imidazolepropionic acid	25.0345	3.8140	26.1507	8.6308	5.7548	8.5836

Table S7 Quantitative results of metabolites from historical silks.

Metabolites (nmol/g)	PBC.Mean	PBC.SD	PBC.Median	PB.Mean	PB.SD	PB.Median
Lysine	840.5457	89.9065	853.9672	355.5816	111.733 ₈	365.2712
Arginine	590.0138	150.6097	536.5507	266.4142	11.2378	266.3447
Glutamine	8.0262	3.7533	6.9031	5.0503	2.2849	6.2746
Glutamic acid	39.5054	6.4976	42.4229	20.1117	4.8173	19.8515
Sarcosine	3.7983	0.2209	3.8902	5.2238	1.7205	6.1796
beta-Alanine	7.2963	1.8712	7.1047	26.5645	4.8762	28.3357
Alanine	207.4349	34.9182	219.8111	341.8744	98.6153	363.2461
Dimethylglycine	1.0573	0.1067	1.0121	7.3462	2.3148	6.4966
GABA	33.9488	25.8582	25.1756	5.2417	1.0889	5.5109
Serine	58.8162	18.5358	52.5318	47.7661	19.9228	58.1603
Threonine	14.9733	3.8631	16.8553	10.6633	4.6294	8.2280
Homoserine	5.7027	0.3939	5.5873	4.6569	1.1237	4.3783
Creatine	3.3591	0.6678	3.2686	3.4946	0.4517	3.3673
Aminocaproic acid	0.9365	0.1900	0.8745	1.8852	0.4319	2.0301
Glycylproline	0.7982	0.0406	0.8099	1.4627	0.2227	1.5615

AMP	0.5367	0.1141	0.4730	0.3926	0.0285	0.3776
Lactic acid	345.5019	97.8659	349.8616	931.3452	422.994 5	1028.9239
Nicotinic acid	12.6814	6.7760	13.3370	2.6368	0.4652	2.5907
Tyrosine	21.6322	5.7708	20.9955	18.4335	2.6726	19.4734
3,4- Dihydroxyhydrocinn amic acid	0.6987	0.0333	0.6974	0.8976	0.0640	0.8756
Asparagine	7.8813	2.3374	7.2739	9.0721	1.1791	9.2391
Protocatechuic acid	57.0373	14.2389	51.1436	58.0681	8.1624	57.6292
Phenylalanine	8.4345	4.6062	6.9398	1.2336	0.9417	0.7332
Hydroxyphenyllactic acid	2.4413	0.2434	2.5462	0.3621	0.1491	0.3301
Glycylleucine	1.5984	0.4139	1.7173	0.4834	0.1020	0.4329
3-Hydroxyhippuric acid	8.5158	1.1593	8.0844	17.7868	3.7209	19.5215
Kynurenine	0.1626	0.2112	0.0407	0.0407	0.0000	0.0407
3-Nitrotyrosine	2.8610	0.6795	3.1206	1.2225	0.3290	1.2168
Aspartic acid	39.1686	7.5764	43.5317	22.4077	10.7168	23.9300
Aminoadipic acid	5.2391	1.4561	5.1874	0.6443	0.9950	0.1288
2-Butenoic acid	6.8126	1.9351	7.0603	4.4328	0.0967	4.4099

Pyrrole-2-carboxylic acid	0.8540	0.1110	0.9086	0.7348	0.1510	0.7580
2-Hydroxy-3-methylbutyric acid	2.3110	0.7946	2.4916	1.9909	0.4248	2.1351
4-Hydroxybenzoic acid	121.1071	11.4885	127.6080	374.8168	45.2682	382.0971
ortho-Hydroxyphenylacetic acid	0.3958	0.0738	0.3745	0.7298	0.0280	0.7421
Hippuric acid	0.3502	0.0430	0.3497	0.5102	0.0894	0.5293
N-Acetyalanine	10.4880	1.0695	10.3069	9.7831	1.8823	9.7390
Malic acid	40.0733	13.4747	37.7158	47.9096	16.7993	52.8840
N-Acetyaspartic acid	2.2126	0.1148	2.2354	2.6704	0.1671	2.5853
Ethylmethylacetic acid	5.8683	2.6262	4.4405	7.9919	2.1133	8.4871
Benzoic acid	42.1703	5.9447	40.2984	121.1190	27.5915	132.7391
2-Hydroxycaproic acid	2.5634	0.2438	2.6981	2.4990	0.9784	3.0018
Phenylacetic acid	20.1593	3.0915	20.1533	63.8892	10.6126	67.3813
Indole-3-carboxylic acid	3.3748	0.6567	3.0978	5.0120	1.1070	5.4459
Coumaric acid/4-Hydroxycinnamic acid	0.2062	0.0284	0.2164	0.1545	0.0846	0.1269
Indoleacetic acid	0.0113	0.0079	0.0089	0.0209	0.0104	0.0214
Fumaric acid	3.1215	0.2722	3.1370	3.3183	0.2002	3.4201

Glutaric acid	8.6134	0.4252	8.7730	25.3177	3.7887	25.5817
Aconitic acid	1.6002	0.3691	1.6658	1.3045	0.3170	1.3732
Cinnamic acid	0.4480	0.1165	0.4842	1.0155	0.1594	1.0200
2-Phenylpropionate	0.3336	0.0458	0.3379	1.2559	0.1884	1.1743
Hydrocinnamic acid	0.3706	0.0629	0.3350	2.4734	0.3385	2.3281
Benzenebutanoic acid	0.3119	0.0345	0.3098	0.5860	0.0650	0.5959
Azelaic acid	43.3844	15.3928	34.9550	43.1574	6.7263	44.3876
Sebacic acid	4.1302	0.7892	4.0100	5.1861	0.5504	5.2309
alpha-Ketoisovaleric acid	4.4569	0.4187	4.2630	5.8274	1.4236	6.2794
Ketoleucine	2.2613	0.5116	2.0519	2.5756	0.2540	2.5084
3-Methyl-2-oxopentanoic acid	1.5857	0.0989	1.6362	2.6535	0.5295	2.7630
Phenylpyruvic acid	1.5474	0.2535	1.4500	2.1841	0.4184	2.1497
Methylmalonic acid	12.3469	1.3315	11.9759	43.2694	5.0661	46.0563
2-Hydroxyglutaric acid	18.2193	2.0125	18.3778	10.7995	1.1271	10.6225
Glycine	196.1454	53.2456	193.0621	248.6763	12.7706	247.0242
Glycolic acid	136.6011	4.4007	136.6066	327.9409	97.9811	378.7902

alpha-Aminobutyric acid	68.8593	2.4821	69.9028	314.7991	75.6066	282.2889
Glyceric acid	18.3724	6.1715	21.2515	45.5272	7.3429	42.0612
Proline	18.9069	8.0457	14.9485	16.2886	4.5202	16.2415
Acetylglycine	5.7383	1.3471	5.7145	12.1549	3.8489	13.7379
Pipecolic acid	0.4738	0.1656	0.4401	0.2727	0.1246	0.2448
Erythronic acid	3.4789	1.5501	3.3773	1.1511	0.3156	1.2212
N-Acetylserine	2.8686	0.6041	3.0527	2.6279	1.0313	2.7005
Acetic acid	2471.0407	494.8492	2420.1847	1675.8626	281.074 5	1767.4816
Valine	799.9822	113.0256	862.0295	3070.9525	678.116 7	3119.7484
Pyroglutamic acid	35.1581	7.9007	33.0106	19.3561	6.4871	20.0982
5-Aminolevulinic acid	2.8561	0.5602	3.0489	5.4827	1.7293	5.9082
Glucose	472.2886	226.9162	424.9339	40.4443	4.2463	40.3156
3-Hydroxybutyric acid	15.5160	4.2377	15.7357	9.7379	2.2891	9.8910
Methionine	0.6885	0.5513	0.8233	2.8326	0.7211	3.1108
2-Hydroxybutyric acid	4.7516	0.6444	4.8418	2.9141	0.9832	3.3264
3-Hydroxyisovaleric acid	9.4560	1.9501	9.0610	7.7302	1.4104	7.0653

Glutaconic acid	7.4730	0.5696	7.4648	10.7109	4.7125	12.6493
Isoleucine	9.8712	3.0360	10.2730	4.5382	3.3652	3.3294
Leucine	10.5126	12.2474	4.0511	0.7145	0.7171	0.6022
Xylose	19.5701	15.6873	13.8191	1.2973	1.6853	0.3243
Ribulose	1.3167	0.1176	1.3822	1.3505	0.0834	1.3815
Xylulose	0.1666	0.2589	0.0171	0.1207	0.0897	0.1710
p-Hydroxymandelic acid	0.1484	0.1250	0.1972	0.1505	0.0777	0.1751
Fructose	6.2003	4.2755	4.2529	0.9713	1.2049	0.3064
Propionic acid	137.5511	25.8000	128.2076	111.5614	15.9000	118.5661
Homovanillic acid	0.2459	0.0456	0.2260	0.2007	0.0649	0.1734
2-Furoic acid	100.1447	4.1516	101.4819	1.3548	0.4914	1.2990
p-Hydroxyphenylacetic acid	0.0343	0.0000	0.0343	0.1372	0.1782	0.0343
Tryptophan	2.5049	0.5085	2.7662	2.2501	0.3440	2.3145
Butyric acid	29.5813	6.8142	26.8393	22.8924	1.0136	22.4258
Isobutyric acid	24.7332	2.5693	24.1988	32.8863	1.1827	32.5584
Mandelic acid	1.2429	0.1375	1.1977	1.7080	0.2117	1.7874

Malonic acid	25.7038	5.9556	28.2859	42.3194	11.2924	47.7552
Valeric acid	81.7758	12.1736	76.6440	90.4411	17.2306	93.9982
N- Methylnicotinamide	16.6607	1.5715	16.6130	54.6899	14.9449	58.3452
Phenyllactic acid	0.2624	0.0874	0.3057	0.2897	0.0729	0.2854
Oxalic acid	257.8487	51.8155	265.6078	1237.6994	$\frac{114.304}{3}$	1217.9829
Maleic acid	2.5789	0.4355	2.3961	2.3007	0.6436	2.5276
Itaconic acid	2.9469	0.1375	2.8738	15.8646	2.8529	15.8444
Citraconic acid	0.1746	0.0739	0.1397	0.2210	0.0275	0.2319
Methylsuccinic acid	7.5965	0.2827	7.5320	24.3750	4.1062	25.7826
Adipic acid	16.8263	5.5798	14.4309	19.7575	3.8418	17.8212
Methylglutaric acid	0.6973	0.1790	0.6965	1.3030	0.2819	1.2751
Phthalic acid	9.8660	1.9196	9.9788	19.6815	2.9374	20.8968
Pimelic acid	5.4390	1.7651	4.4389	8.4057	0.8446	8.4158
3-Methyladipic acid	0.3398	0.0844	0.3770	0.6991	0.0736	0.6821
2-Methylpentanoic acid	1.1307	0.2410	1.1117	0.9641	0.3498	1.0869
Caproic acid	130.5495	9.4931	128.4203	155.3902	24.0475	154.1939

Suberic acid	15.4022	4.8730	12.7874	16.1139	1.8219	15.4560
Citric acid	6.6844	3.7333	7.4214	2.1453	0.6844	2.0342
Isocitric acid	6.8820	2.6705	7.4425	15.5925	10.7925	18.3873
Heptanoic acid	39.0806	0.9047	38.8370	78.7891	10.2189	73.0046
Pyruvic acid	28.4838	8.0004	27.0030	92.4203	19.1328	101.4625
7-DHCA	0.0534	0.0169	0.0477	0.1062	0.0390	0.1255
Oxoglutaric acid	0.8897	0.1408	0.9422	0.8107	0.1326	0.7474
Octanoic acid	374.2079	26.7644	367.8052	1572.8603	$\frac{261.944}{6}$	1540.4905
CA	0.0732	0.0079	0.0722	0.5483	0.3786	0.4363
Oxoadipic acid	0.1613	0.0333	0.1503	0.1294	0.0327	0.1287
Decanoic acid	71.6732	9.9429	73.5303	247.0517	72.2059	284.0712
Undecylenic acid	0.5784	0.1600	0.4926	1.8303	0.4784	1.8296
Undecanoic acid	8.1232	0.4016	8.1171	32.5022	10.5995	35.6467
12-Tridecenoic acid	0.6125	0.1577	0.5694	1.0419	0.3286	1.1545
Tridecanoic acid	8.3518	0.9763	8.5712	23.9526	8.1451	28.4287
Myristoleic acid	1.2336	0.1253	1.2115	0.6549	0.1214	0.5954

9E-tetradecenoic acid	14.1193	2.6819	13.0402	8.6114	1.9678	9.1239
Ricinoleic acid	8.5244	2.9349	7.6003	7.0110	1.0349	6.8852
12-Hydroxystearic acid	10.3483	1.0583	9.8647	35.5664	7.2759	38.9688
LCA	0.0848	0.0208	0.0928	0.2502	0.0005	0.2501
Myristic acid	60.5795	4.7811	63.0768	100.6692	17.7141	98.0281
9-Pentadecenoic acid	0.8124	0.3714	0.6001	0.8320	0.1523	0.8251
Pentadecanoic acid	40.1484	3.2302	41.7262	36.7921	4.3295	37.2626
Palmitoleic acid	26.5564	5.6928	25.0864	22.6481	6.6459	23.3681
Palmitelaidic acid	10.4796	2.2781	11.3789	12.2936	1.9637	12.1950
10Z-Heptadecenoic acid	3.2613	0.1873	3.3132	1.2384	0.1263	1.2560
10-Trans-Heptadecenoic acid	3.8269	0.3416	3.7613	1.4034	0.1913	1.4968
alpha-Linolenic acid	9.9163	3.9188	11.0975	0.4693	0.0488	0.4927
Linoleic acid	764.6751	273.4748	901.3633	44.9380	6.9654	45.0431
Succinic acid	36.2940	1.8281	36.2960	142.0175	26.1326	156.3370
Citramalic acid	2.0524	0.3284	1.9415	15.4969	10.2712	14.9492
10Z-Nonadecenoic acid	0.4631	0.0978	0.4211	0.3250	0.0351	0.3435

Carnitine	23.6679	12.2061	20.3266	0.1971	0.2580	0.0910
Acetylcarnitine	0.5431	0.0269	0.5532	0.4082	0.0417	0.3870
Propionylcarnitine	0.0188	0.0037	0.0195	0.0090	0.0002	0.0089
Butyrylcarnitine	0.0232	0.0081	0.0258	0.0149	0.0009	0.0154
2-Methylbutyrylcarnitine	0.1157	0.0000	0.1157	0.1157	0.0000	0.1157
Valerylcarnitine	0.0184	0.0021	0.0178	0.0118	0.0011	0.0116
Isovalerylcarnitine	0.0204	0.0070	0.0180	0.0141	0.0030	0.0139
3-Hydroxyisovalerylcarnitine	0.0182	0.0009	0.0178	0.0078	0.0005	0.0078
Norvaline	15.1742	6.5058	14.8158	12.9332	3.5247	11.6003
2,2-Dimethyladipic acid	1.9418	0.1783	2.0421	7.0244	1.0874	7.6051
12-KetoLCA	0.2805	0.0304	0.2669	0.2949	0.0395	0.2915
Indole-3-carboxaldehyde	3.4700	0.5369	3.7407	7.8236	1.0746	7.9217
2,2-Dimethylsuccinic acid	0.3451	0.2506	0.4763	1.0649	0.3642	1.1852
Indolelactic acid	0.2401	0.0383	0.2511	0.0674	0.0101	0.0672
6,7-DiketoLCA	1.6753	0.1453	1.6165	1.3879	0.5081	1.1438
gamma-Glutamylalanine	0.8946	0.2681	0.8989	1.1696	0.1484	1.1710

Imidazolepropionic acid	0.2435	0.0383	0.2335	0.2929	0.1471	0.2844
Threonic acid	2.7290	1.2275	3.0609	1.1490	0.3300	0.9769
N-Acetylserotonin	0.2420	0.0931	0.2248	0.4362	0.1066	0.3937
Hydroxypropionic acid	228.8697	42.7884	208.2183	545.5593	129.216 5	612.8025
Gallic acid	2.5287	0.3007	2.4467	2.8344	0.4912	2.9818
3-Chlorotyrosine	8.9110	2.0905	8.0354	12.4332	2.1424	12.3150
Glucaric acid	0.8308	0.2024	0.8687	0.6786	0.1826	0.7702
2-Methy-4-pentenoic acid	0.9579	0.1255	0.9605	0.9348	0.3437	0.9942
Indoleacrylic acid	0.6750	0.1869	0.5881	0.5393	0.1283	0.5565
Indole-3-propionic acid	0.1231	0.0068	0.1248	0.1153	0.0077	0.1114
Maltose/Lactose	11.6949	4.8784	12.9112	0.4263	0.2568	0.4359
Glyceraldehyde	1.0180	1.2745	0.5373	21.6926	6.0671	24.5383
Norleucine	0.0529	0.0000	0.0529	0.0529	0.0000	0.0529
3-Phenylbutyric acid	0.0722	0.0299	0.0736	0.3346	0.1924	0.2703
4-Methylhexanoic acid	1.6779	0.1312	1.6835	1.3899	0.0433	1.3681
5Z-Dodecenoic acid	0.2384	0.0200	0.2494	0.4422	0.0555	0.4511

Oleic acid	271.0330	39.7211	289.2895	100.7552	15.3574	99.8043
Hexanoylcarnitine	0.0182	0.0066	0.0147	0.0037	0.0004	0.0039
Octanoylcarnitine	0.0173	0.0031	0.0170	0.0042	0.0003	0.0042
Dodecanoylcarnitine	0.0071	0.0005	0.0071	0.0072	0.0001	0.0072
Tetradecanoylcarnitine	0.0040	0.0001	0.0040	0.0025	0.0003	0.0026
Palmitoylcarnitine	0.0115	0.0027	0.0113	0.0075	0.0009	0.0074
Oleylcarnitine	0.0069	0.0002	0.0070	0.0064	0.0011	0.0061
Stearyl carnitine	0.0151	0.0036	0.0143	0.0127	0.0004	0.0125

Reference

1. D. Badillo-Sanchez, D. Chelazzi, R. Giorgi, A. Cincinelli and P. Baglioni, *Sci Rep*, 2019, **9**, 10.
2. M. McGill, G. P. Holland and D. L. Kaplan, *Macromol. Rapid Commun.*, 2019, **40**, 14.
3. N. Qin, S. Q. Zhang, J. J. Jiang, S. Gilbert Corder, Z. G. Qian, Z. T. Zhou, W. Lee, K. Y. Liu, X. H. Wang, X. X. Li, Z. F. Shi, Y. Mao, H. A. Bechtel, M. C. Martin, X. X. Xia, B. Marelli, D. L. Kaplan, F. G. Omenetto, M. K. Liu and T. H. Tao, *Nat. Commun.*, 2016, **7**, 8.
4. S. J. Ling, Z. M. Qi, D. P. Knight, Z. Z. Shao and X. Chen, *Biomacromolecules*, 2011, **12**, 3344-3349.
5. J. D. van Beek, L. Beaulieu, H. Schafer, M. Demura, T. Asakura and B. H. Meier, *Nature*, 2000, **405**, 1077-1079.
6. D. Badillo-Sanchez, D. Chelazzi, R. Giorgi, A. Cincinelli and P. Baglioni, *Polym. Degrad. Stabil.*, 2018, **157**, 53-62.
7. T. Asakura, *Molecules*, 2021, **26**, 20.
8. T. Shu, J. Cui, Z. C. Lv, L. T. Cao, J. Ren and S. J. Ling, *Soft Matter*, 2021, **17**, 9579-U9512.