Supporting Document

Identification and bioactivity evaluation of flavan-3-ols in the milk of dairy sheep fed *Cynomorium songaricum*

Shen Duan,[1,2] Xin-Yao Wang,[1,2] Xu Feng,[1,2] Yue Ma,[1,2] Zhong Zheng,[1] Chao-Mei Ma[1,2]*

[1]State Key Laboratory of Reproductive Regulation & Breeding of Grassland Livestock, School of Life Sciences, Inner Mongolia University, Hohhot, PR China 010070.

[2]Key Laboratory of Herbage and Endemic Crop Biology of Ministry of Education, School of Life Sciences, Inner Mongolia University, Hohhot, PR China 010070.

*Corresponding author: cmma@imu.edu.cn

Table S1 The optimized MRM condition in UHPLC-MS for quantification of the flavan-3-ols

Compound Name	Molecular weight	Precursor ion (m/z)	Product Ion(m/z)	Fragmentor (V)	Collision energy (eV)	Polarity
Catechin	290	289	123	130	28	N
Epicatechin	290	289	123	130	28	N
Procyanidin A-1	576	575	449	150	14	N
Procyanidin A-2	576	575	449	150	14	N
Procyanidin B-1	578	577	289	90	20	N

Table S2 UHPLC–ESIMS analytical parameters for quantification of flavan-3-ols in the sheep milk.

compound	Retention Time (min)	Calibration Equation[1]	Calibration Range (nM)	LOD (nM)	LOQ (nM)	RSD[2]	RSD[3]	Accuracy (%)
Catechin	1.255	[4]y=2245300.75x-0.32	169.55-10813.15	83.05	169.55	2.60	10.31	92.0-116.3
Epicatechin	2.276	y=1662913.21x+5.17	169.55-2712.80	83.05	169.55	2.79	8.59	99.7-111.1
Procyanidin A-1	2.318	y=524909.90x+0.62	85.21-340.87	41.74	85.22	3.65	9.94	81.5-113.2
Procyanidin A-2	4.124	y=1254509.20x+0.19	1000.00-21739.13	500.00	1000.00	5.13	7.49	90.5-101.1
Procyanidin B-1	1.060	y=5179722.70x+19.61	500.00-5000.00	249.91	500.00	10.25	8.81	95.5-112.6

[1]$R^2 > 0.99$.

[2]Intra-day precision from 3 determinations. [3]Inter-day precision from 3 consecutive days.

[4]y, chromatogram peak area; x, compound concentration in nM.

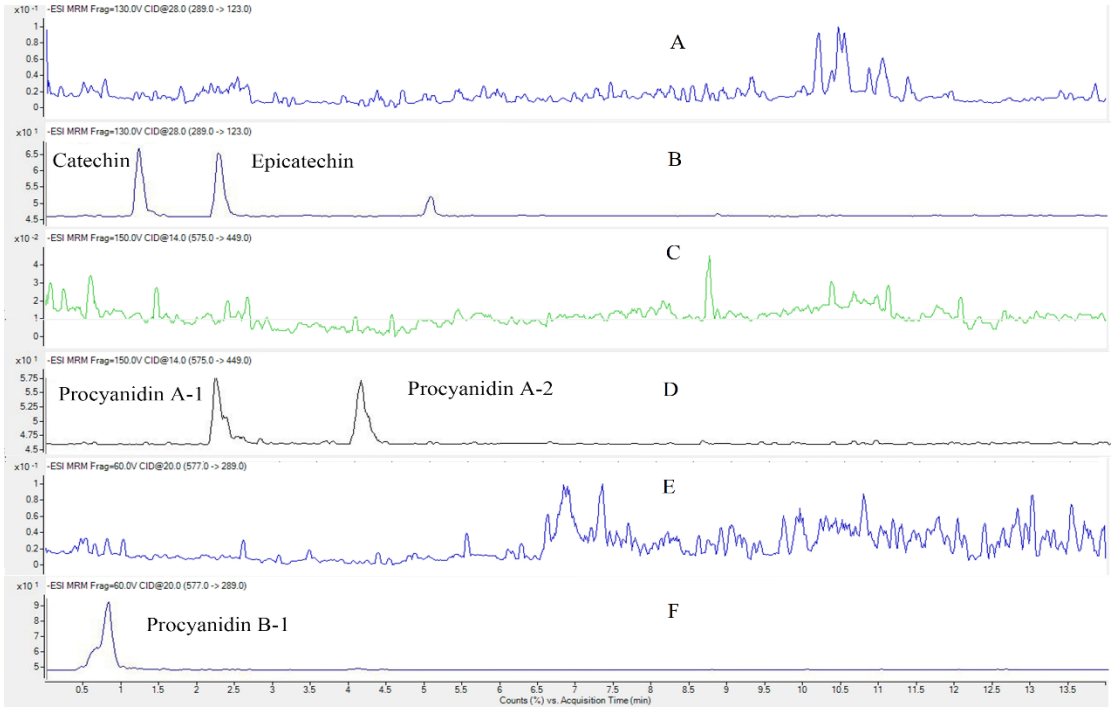

Figure S1 Chromatograms of UHPLC-MS in MRM mode of the flavan-3-ols in the regular TMR for dairy sheep in this experimental base. A, C and E are the chromatograms of the TMR extract, and B, D, and F are those of standard compounds.

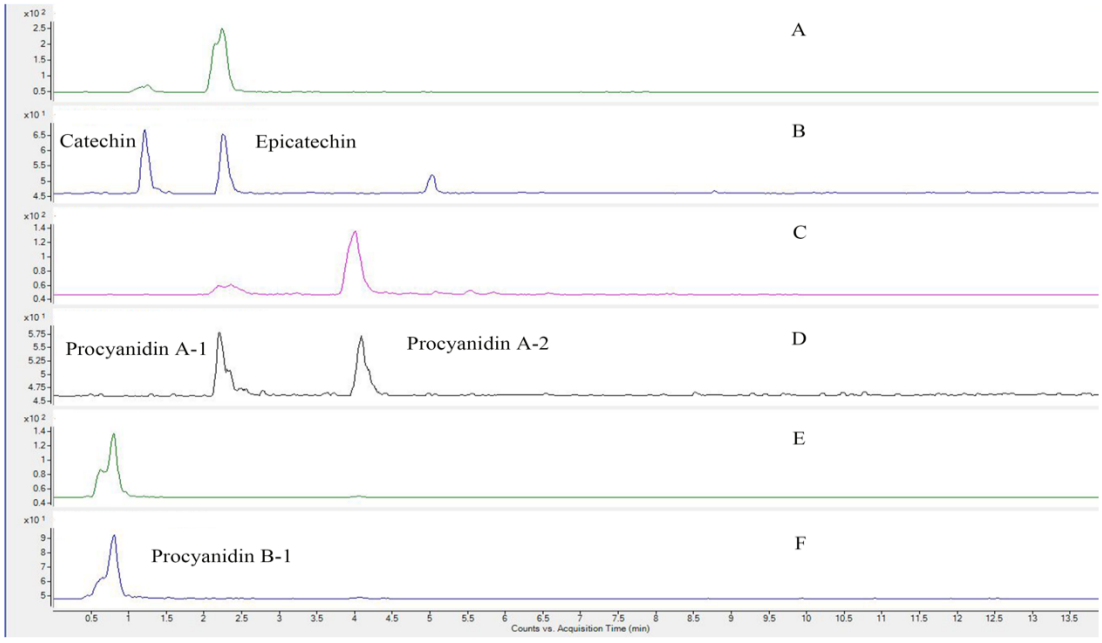

Figure S2 Chromatograms of UHPLC-MS in MRM mode of flavan-3-ols in the dairy sheep milk collected at 24h after being fed with *C. songaricum*. A, C and E are the chromatograms of sheep milk, and B, D, and F are those of standard compounds.

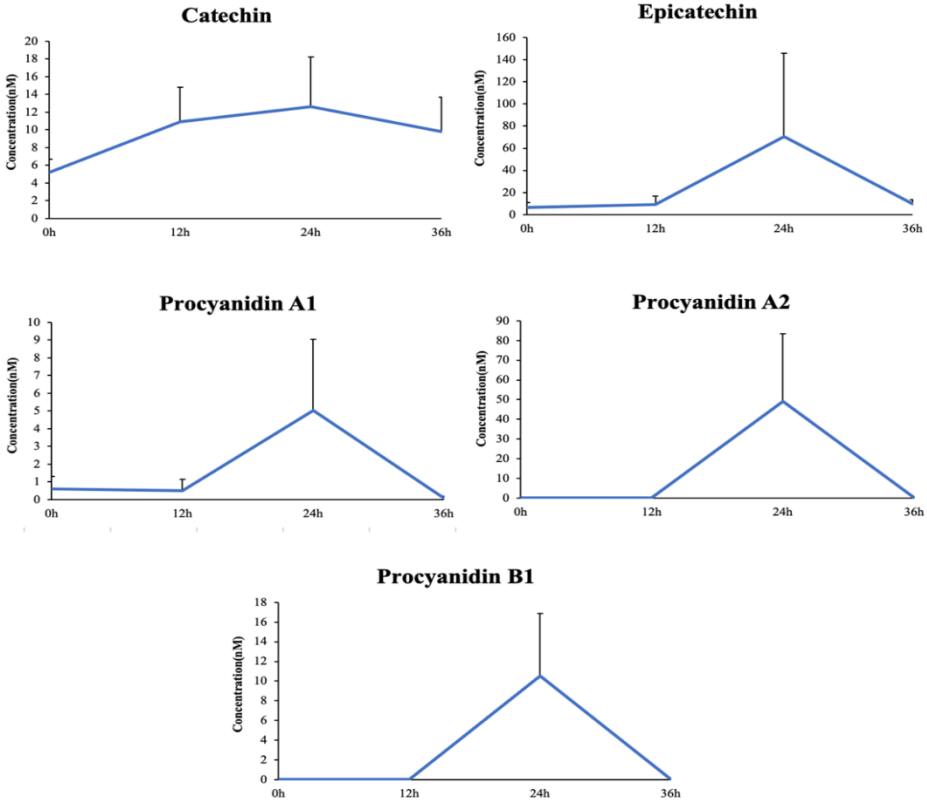

Figure S3 Concentrations *v. s.* time curves of flavan-3-ols in the milk of sheep fed *C. songaricum.*

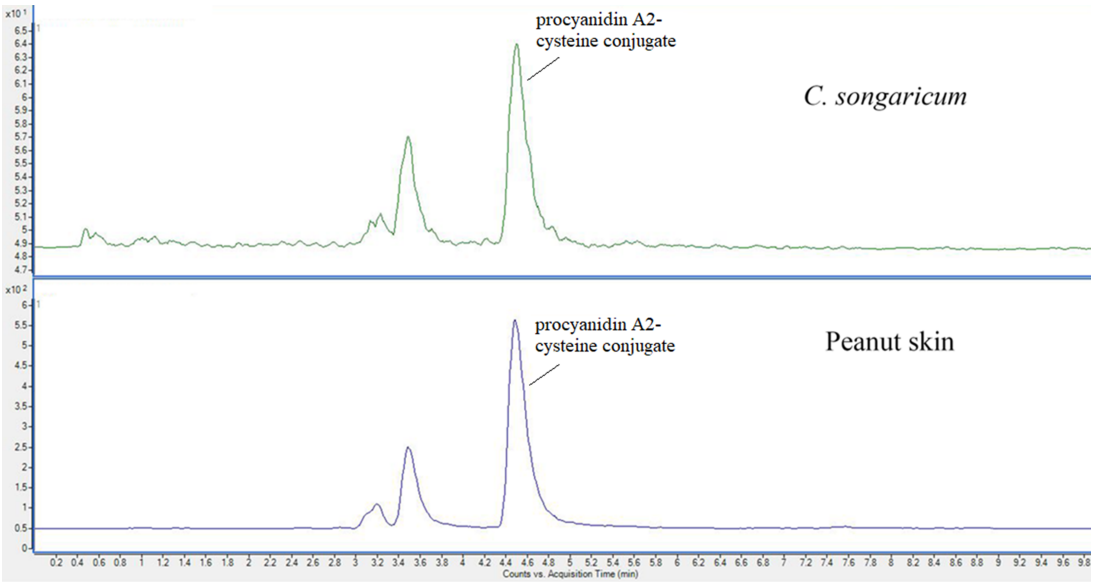

Figure S4 UHPLC-MS chromatograms of the cysteine degradation products of *C. songaricum* and peanut skin. UHPLC-(-)MS in MRM mode with precursor ion 694 and product ion 573 targeting at procyanidin A-cysteine conjugates.

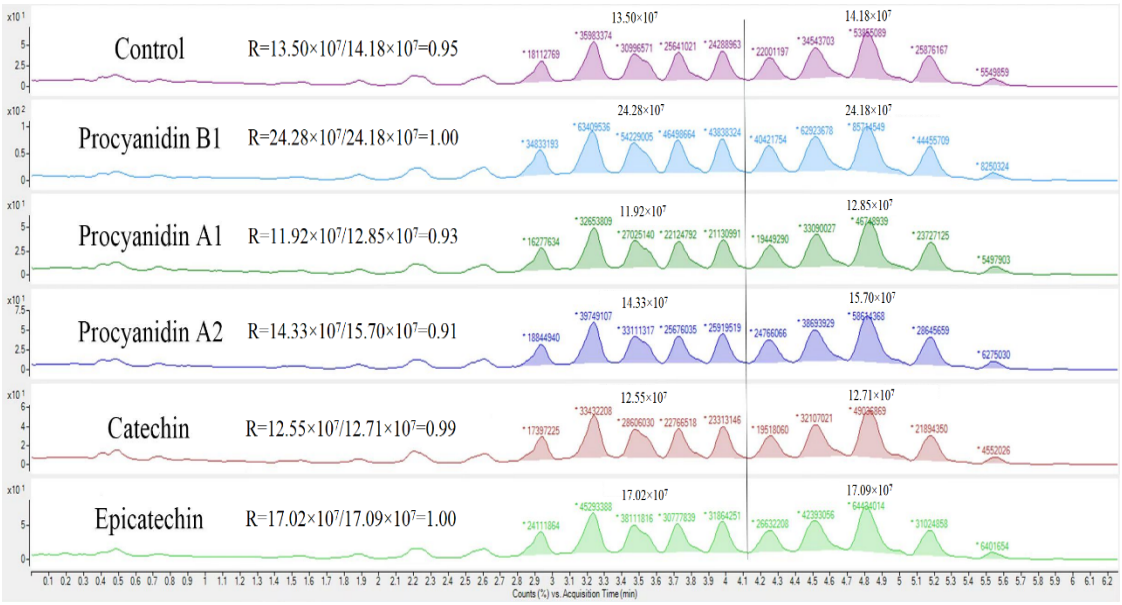

Figure S5 UHPLC-APCIMS chromatograms of triglycerides in the milk after accelerated oxidation. From upper to lower: blank milk, procyanidin B1-containing milk, procyanidin A1-containing milk, procyanidin A2-containing milk, catechin-containing milk, and epicatechin-containing milk. The integral areas on the top of each peak were automatically calculated and drawn by the UHPLC-MS instrument. The values written in black are the area sums of chromatograph peaks with retention times less than 4.28 (left of the vertical line) and that with retention times equal to and larger than 4.28 (right of the vertical line).

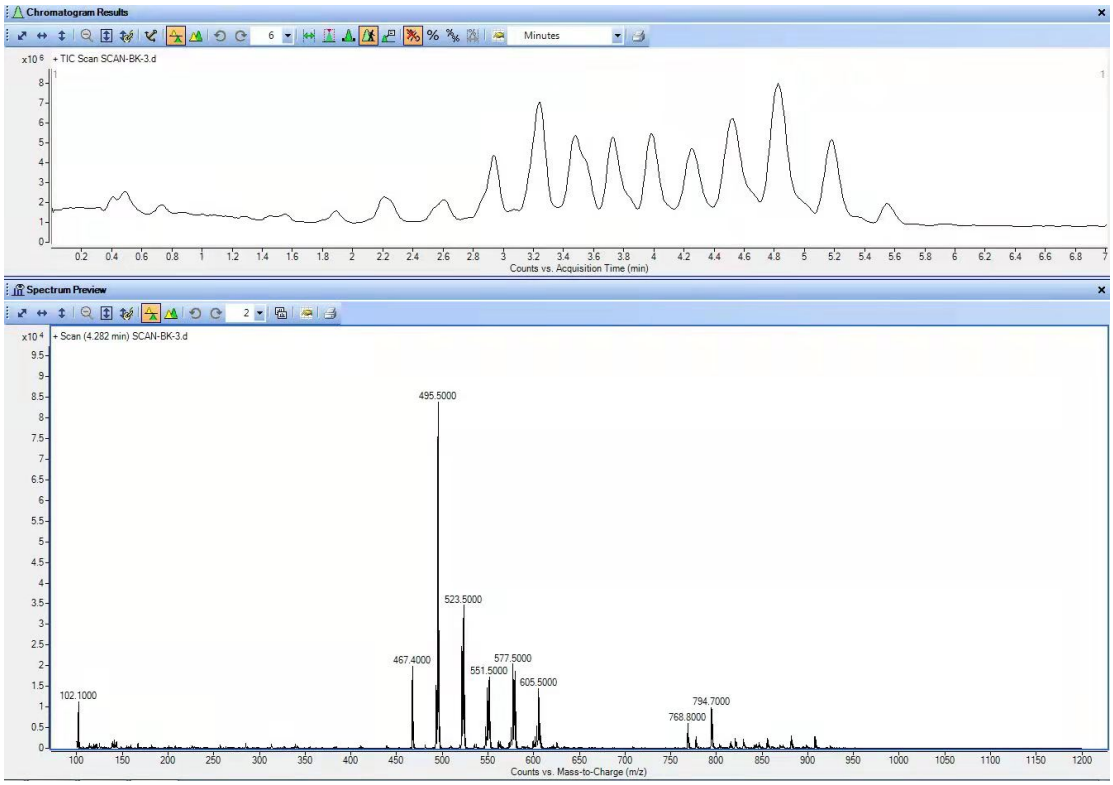

Figure S6 UHPLC-APCIMS of triglycerides in the milk after accelerated oxidation. Upper: UHPLC-APCIMS chromatogram of blank milk; Lower: MS spectrum of the peak at 4.28 min contains 4 triglycerides all of which have 44 of equivalent carbon numbers. These triacyglycerols include SPC (Stearic, Palmitic and Capric acid), SMLa (Stearic, Myristic and Lauric acid), OMM (Oleic, Myristic and Myristic acid), and OPLa (Oleic, Palmitic and Lauric acid). SPC that contains fatty acids of 18:0/16:0/10:0 displayed $[M+NH_4]^+$ at 768.8 and product ions at 467.4 $[M+H-S]^+$ and 579.5 $[M+H-C]^+$. SMLa that contains fatty acids of 18:0/14:0/12:0 displayed $[M+ NH_4]^+$ at 768.8 and product ions at 467.4 $[M+H-S]^+$ and 551.5 $[M+H-La]^+$. OMM that contains fatty acids of 18:1/14:0/14:0 displayed $[M+NH_4]^+$ at 794.7 and product ions at 495.5 $[M+H-O]^+$ and 549.5 $[M+H-M]^+$. OPLa that contains fatty acids of 18:1/16:0/12:0 displayed $[M+NH_4]^+$ at 794.7 and product ions at 495.5 $[M+H-O]^+$ and 577.5 $[M+H-La]^+$.