

## Supporting Information

For the article

### **Salivary cues: Simulated roe deer browsing induces systemic changes in phytohormones and defense chemistry in wild-grown maple and beech saplings.**

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#### **The following Supporting Information is available for this article:**

**Figure S1** Changes in bud and leaf phytohormones two days after simulated browsing.

**Figure S2** LC-UV chromatograms showing most abundant defense metabolites in lateral buds.

**Figure S3** LC-UV chromatograms showing most abundant defense metabolites in subapical leaves.

**Table S1** Cytokinin concentration (tZ, tZR, DHZR, IPR) in buds after simulated browsing.

**Table S2** Concentration of major phenolic compounds after simulated browsing as analyzed by LC-UV-DAD chromatography.

**Table S3** Relative quantification (peak area) of minor phenolic compounds (pathway intermediates and compounds with lower abundance) after simulated browsing as analyzed by LC-MS/MS chromatography.

**Table S4** Soluble sugars in buds and leaves after simulated browsing.

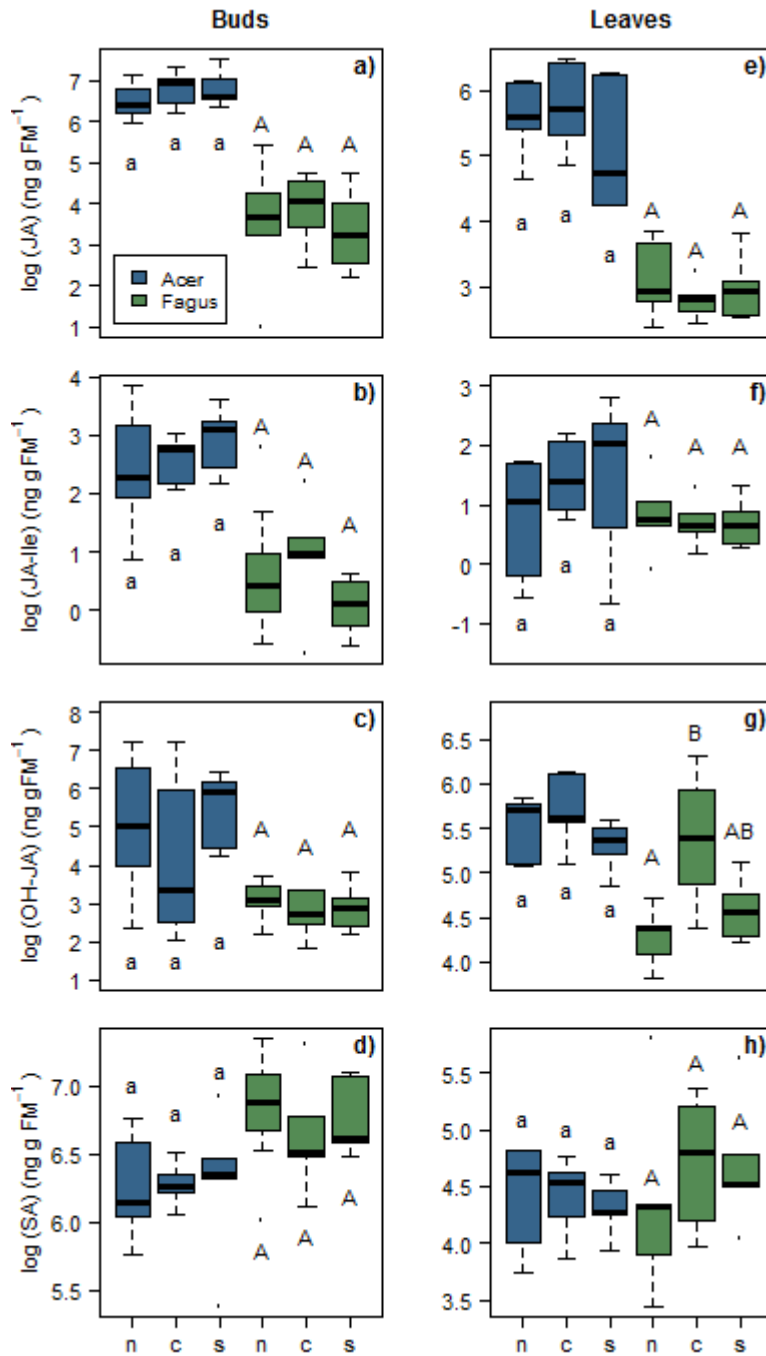
**Table S5** Free amino acids in buds and leaves after simulated browsing.

**Methods S1** Internal standards used for the analysis of phytohormones

**Methods S2** LC-UV-DAD chromatography for analysis of abundant phenolics

**Methods S3** LC-MS/MS chromatography for analysis of minor phenolic compounds

**Methods S4** Sugar and amino acid analyses



**Figure S1** Changes in bud and leaf phytohormones of *Acer pseudoplatanus* and *Fagus sylvatica* two days after simulated browsing. a) and e) JA – Jasmonic acid; b) and f) JA-Ile – Jasmonic acid Isoleucine; c) and g) OH-JA – Hydroxy-Jasmonic acid; d) and h) SA – Salicylic acid. Treatments: n – none, c – clipping, s – saliva application in addition to clipping. Significant differences (multiple comparison Kruskal-Wallis test,  $p < 0.05$ ) between treatments within each species are indicated by different letters (*Acer pseudoplatanus* in lower case, *Fagus sylvatica* in upper case).

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**Methods S1** Internal standards used for the analysis of phytohormones:

Jasmonic acid (JA) and Hydroxy-Jasmonic acid (OH-JA): D6-jasmonic acid, HPC Standards GmbH, Cunnersdorf, Germany, 40ng per sample; Jasmonic acid-Isoleucine (JA-Ile): Jasmonic acid-13C6-isoleucine, 8ng per sample; Salicylic acid (SA): D4-salicylic acid, Sigma-Aldrich, 40ng per sample; trans-Zeatin (tZ): D5-trans-Zeatin, Olchemin, Czech Republic, 1ng per sample; trans-Zeatin-Riboside (tZR) and Dihydrozeatin-Riboside (DHZR): D5-trans-Zeatin-Riboside, Olchemin, Czech Republic, 0.1ng per sample; Isopentenyladenenin-Riboside (IPR): D6-Isopentenyladenenin-Riboside Olchemin, Czech Republic, 0.2ng per sample.

**Table S1** Cytokinins (ng gFM<sup>-1</sup>) in *Acer pseudoplatanus* and *Fagus sylvatica* buds two hours (h) and two days (d) after simulated browsing. tZ – trans-Zeatin; tZR – trans-Zeatin-Riboside; DHZR – Dihydrozeatin-Riboside; IPR – Isopentenyladenenin-Riboside.

Cytokinin	Time	No treatment		Clipping		Clipping & Saliva	
		Mean	SD	Mean	SD	Mean	SD
<i>Acer pseudoplatanus</i>							
tZ	h	0.99	0.54	1.27	0.77	1.52	0.59
	d	0.63	0.45	3.27	1.64	3.95	0.63
tZR	h	1.11	1.05	0.87	0.22	0.95	0.75
	d	0.46	0.34	1.96	0.89	2.33	0.35
DHZR	h	0.17	0.07	0.10	0.07	0.13	0.13
	d	0.17	0.22	0.15	0.03	0.11	0.04
IPR	h	0.91	0.78	0.64	0.25	0.63	0.42
	d	0.41	0.21	0.87	0.55	0.98	0.43
<i>Fagus sylvatica</i>							
tZ	h	1.80	1.17	1.41	0.56	1.01	0.57
	d	1.73	0.33	1.87	0.29	2.70	0.85
tZR	h	1.19	0.73	0.74	0.40	0.69	0.13
	d	0.89	0.51	1.37	0.67	1.96	0.57
DHZR	h	0.22	0.32	0.09	0.06	0.08	0.04
	d	0.12	0.13	0.14	0.09	0.14	0.06
IPR	h	0.12	0.08	0.12	0.09	0.11	0.05
	d	0.10	0.03	0.13	0.10	0.15	0.04

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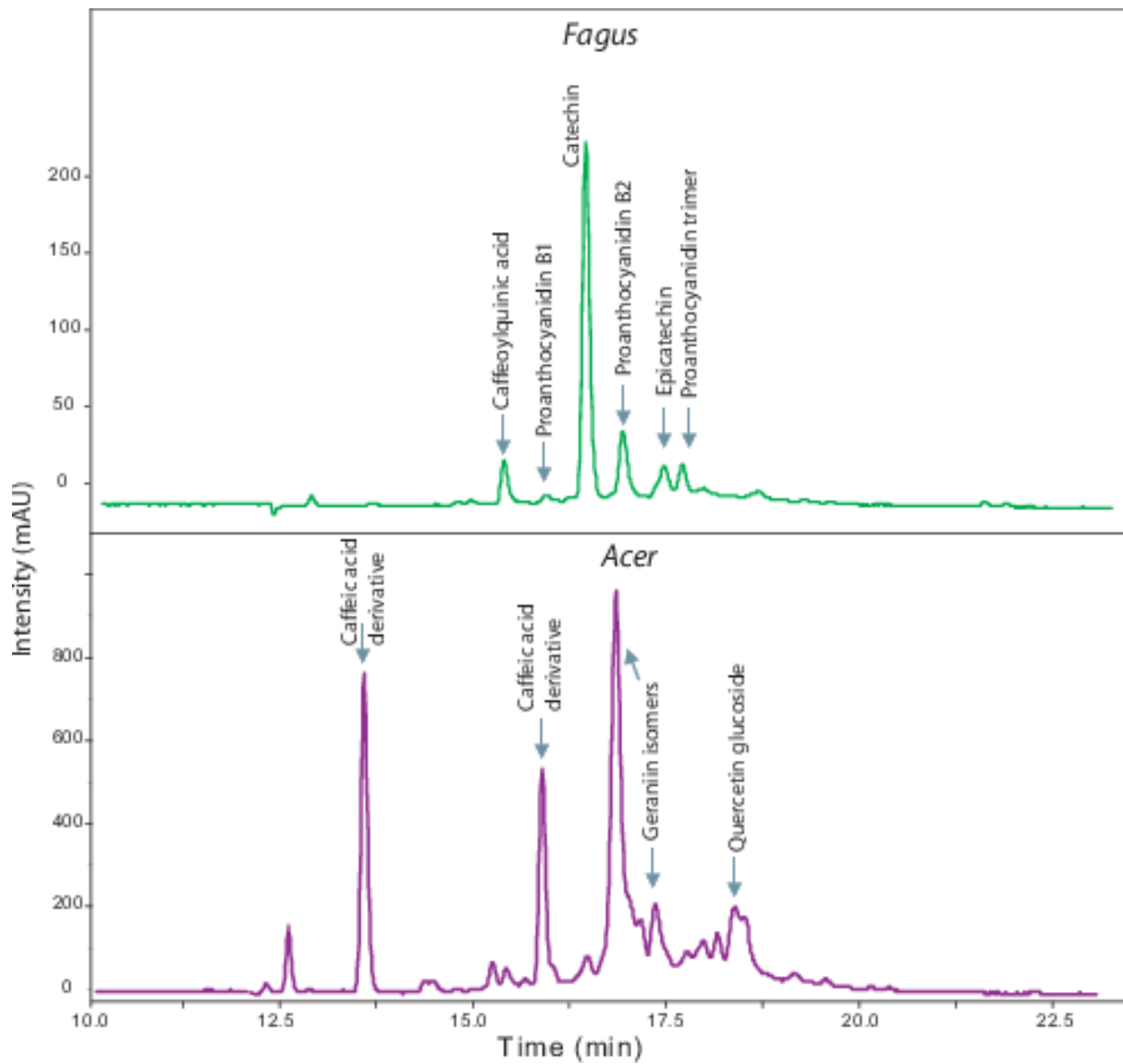
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## **Methods S2**

LC-UV-DAD chromatography was performed on an Agilent 1100 HPLC system (Agilent Technologies, Boeblingen, Germany) with a flow rate of 1.0 ml min<sup>-1</sup>. Separation was achieved on a Nucleodur Sphinx RP18ec column with dimensions of 250 x 4.6 mm and a particle size of 5 µm (Macherey-Nagel, Dueren, Germany). The column temperature was maintained at 20°C. 0.2% (v/v) formic acid in water and acetonitrile were employed as mobile phases A and B, respectively. The elution profile was: 0-5 min 100% A, 5-20 min, 0-45% B in A; 20-22 min, 100% B and 22-26 min, 100% A. UV signals of 200nm, 240nm, 260nm, 280nm and 330nm were monitored. Chromatograms recorded at 280nm were used for integration and quantification. External standard curves generated by dilution series of authentic standards were used to quantify the following chromophore equivalents: quercetin for quercetin, quercetin glucoside and quercetin rhamnoside; kaempferol for kaempferol rhamnoside and kaempferol glucoside; gallic acid for geraniin, corilagin, unknown ellagitannins and maplexin G; catechin for catechin, epicatechin, proanthocyanidin B1 and B2 and proanthocyanidin trimer; chlorogenic acid for caffeoylquinic acid and unknown caffeic acid derivative.

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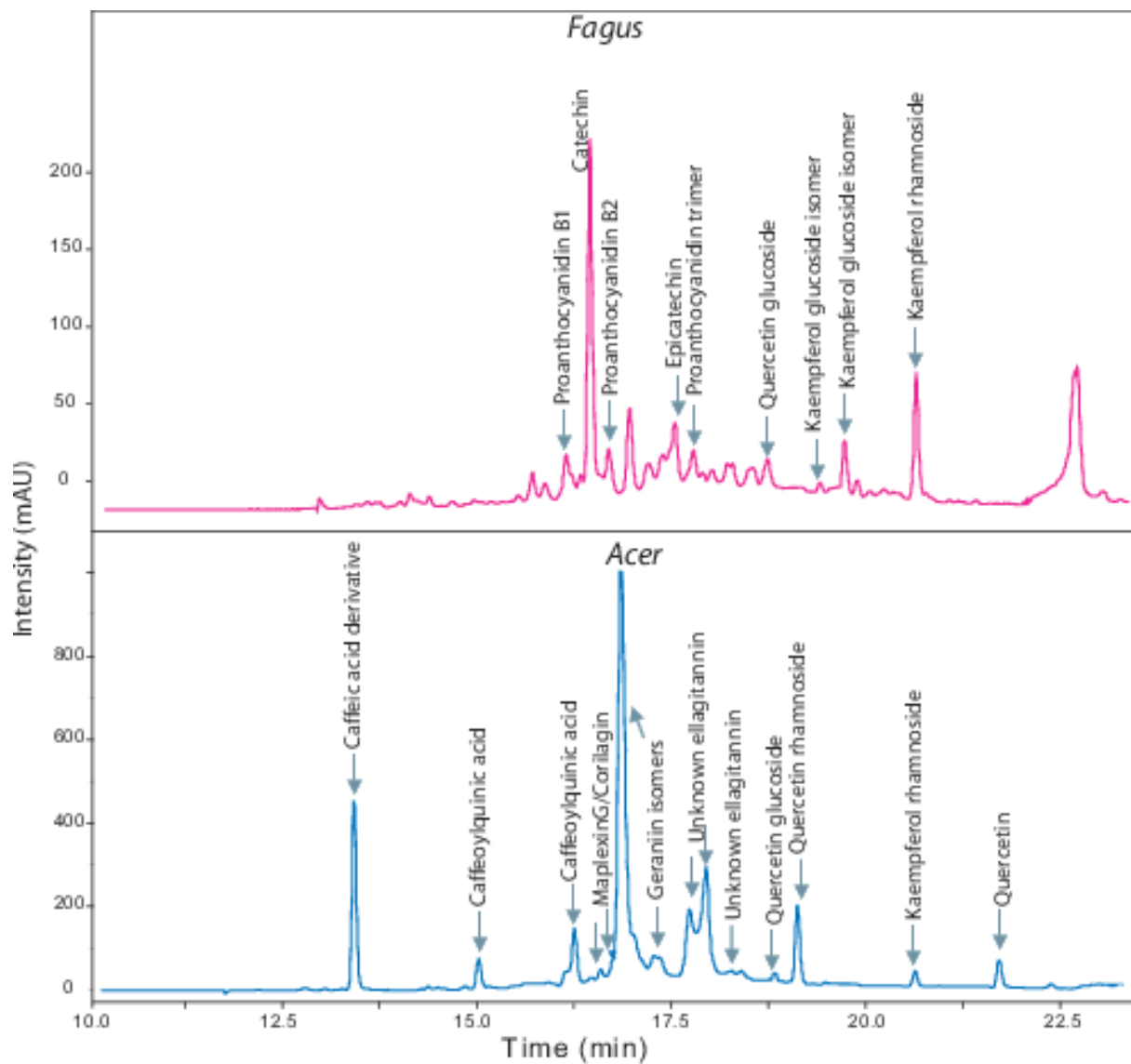
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**Figure S2** LC-UV chromatograms at 280nm, showing the most abundant defense metabolites in lateral buds of *Fagus sylvatica* (top) and *Acer pseudoplatanus* (bottom).

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**Figure S3** LC-UV chromatograms at 280nm, showing the most abundant defense metabolites in subapical leaves of *Fagus sylvatica* (top) and *Acer pseudoplatanus* (bottom).

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**Table S2** Concentration ( $\mu\text{g mgFM}^{-1}$ ) of major phenolic compounds in *Acer pseudoplatanus* and *Fagus sylvatica* buds and leaves two hours (h) and two days (d) after simulated browsing. RT – retention time (minutes). MW – molecular weight. Data based on LC-UV-DAD analyses.

RT	Phenolic compound	Time	No treatment		Clipping		Clipping & Saliva	
			Mean	SD	Mean	SD	Mean	SD
<b>Buds</b>								
<i>Acer pseudoplatanus</i>								
13.6	Caffeic acid derivative1 (MW 344)	h	4.50	1.32	4.40	1.70	3.24	1.28
		d	5.58	2.00	5.06	1.48	6.46	1.77
15.9	Caffeic acid derivative2 (MW 360)	h	4.19	1.11	3.39	0.68	3.64	1.33
		d	2.72	1.14	2.42	0.75	3.19	2.42
17.3	Geraniin isomer (MW 952)	h	23.55	10.08	20.11	6.57	16.36	4.67
		d	23.45	18.08	16.99	6.19	24.60	6.88
<i>Fagus sylvatica</i>								
15.2	Caffeoylquinic acid isomer 1	h	0.89	0.58	0.42	0.33	0.58	0.16
		d	0.76	0.43	0.61	0.25	0.70	0.21
15.8	ProanthocyanidinB1	h	1.64	0.38	0.80	0.63	1.04	0.18
		d	1.11	0.16	1.14	0.20	1.43	0.36
16.4	Caffeoylquinic acid isomer 2	h	3.15	1.20	2.43	0.79	1.83	0.45
		d	5.35	2.40	3.74	1.69	5.15	3.01
16.8	Catechin	h	3.77	1.40	2.01	0.68	2.88	0.73
		d	2.98	0.82	3.15	0.80	3.88	0.45
17.1	ProanthocyanidinB2	h	2.20	0.91	2.34	2.54	1.53	0.46
		d	2.50	2.47	1.77	0.41	2.13	0.81
17.8	Proanthocyanidin trimer	h	3.45	1.23	2.37	0.84	2.15	0.78
		d	3.65	1.21	2.96	0.77	3.74	1.56
<b>Leaves</b>								
<i>Acer pseudoplatanus</i>								
13.3	Caffeic acid derivative (MW 344)	h	1.16	1.52	0.92	0.72	1.43	0.76
		d	1.33	1.17	0.97	0.74	0.42	0.19
15.0	Caffeoylquinic acid isomer 1	h	0.39	0.19	0.28	0.13	0.38	0.13
		d	0.15	0.11	0.32	0.16	0.34	0.09
16.2	Caffeoylquinic acid isomer 2	h	0.86	0.16	0.76	0.27	0.85	0.13
		d	0.43	0.25	0.72	0.46	0.95	0.06
16.6	MaplexinG (MW 634)	h	1.38	0.56	1.23	0.24	1.39	0.49
		d	0.90	0.61	1.18	0.25	1.79	0.47
16.9	Corilagin (MW 634)	h	1.78	0.91	1.49	0.50	1.90	0.85
		d	1.21	0.85	1.63	0.42	1.94	0.36
17.1	Geraniin isomer1 (MW 952)	h	24.53	13.62	18.66	6.51	20.63	6.92
		d	15.64	9.70	21.81	3.98	31.00	8.48
17.3	Geraniin isomer2 (MW 952)	h	5.26	2.17	4.67	1.60	6.16	2.54
		d	3.44	2.10	4.99	0.37	6.54	1.63
17.7	Unknown Ellagitannin1 (MW 950)	h	6.99	3.05	6.13	1.83	5.71	1.71

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		d	5.61	3.89	6.90	1.99	9.78	3.63
18.2	Unknown Ellagitannin2 (MW 950)	h	5.65	4.05	4.14	2.39	3.97	1.87
		d	5.74	4.51	4.84	2.66	9.74	4.78
18.4	Unknown Ellagitannin3 (MW 950)	h	6.49	4.81	6.58	3.34	5.23	3.46
		d	8.20	6.29	7.23	5.09	13.02	7.03
18.8	Quercetin glucoside	h	3.09	2.51	2.67	0.71	3.14	2.00
		d	2.63	1.91	1.44	1.38	5.08	2.22
19.6	Quercetin rhamnoside	h	4.13	2.02	3.33	1.77	4.35	1.38
		d	1.44	0.87	4.58	1.54	5.40	1.93
20.6	Kaempferol rhamnoside	h	0.86	0.38	0.70	0.27	0.78	0.12
		d	0.38	0.23	0.87	0.44	1.13	0.51
21.3	Quercetin	h	0.66	0.71	0.50	0.78	0.21	0.34
		d	0.39	0.37	1.02	0.73	0.59	0.66

*Fagus sylvatica*

15.5	ProanthocyanidinB1	h	0.90	0.49	0.82	0.09	0.84	0.39
		d	1.06	0.20	1.09	0.54	1.17	0.42
15.9	Catechin	h	4.56	0.71	4.35	1.48	3.97	0.76
		d	6.61	1.70	5.40	2.21	6.53	1.02
16.2	ProanthocyanidinB2	h	0.85	0.36	0.93	0.32	0.89	0.36
		d	1.09	0.63	1.26	0.62	0.82	0.34
17.3	Epicatechin	h	0.89	0.85	0.52	0.65	0.69	0.86
		d	0.99	1.04	0.88	0.67	1.03	0.62
17.4	Proanthocyanidin trimer1	h	1.31	0.58	1.16	0.30	0.67	0.64
		d	1.52	0.18	1.30	0.31	1.65	0.51
18.0	Proanthocyanidin trimer2	h	0.84	0.61	0.47	0.28	0.72	0.17
		d	0.86	0.41	0.90	0.55	0.80	0.66
18.7	Quercetin glucoside	h	1.09	0.97	0.84	0.22	1.01	0.41
		d	1.03	0.52	1.08	0.78	1.25	0.58
19.1	Kaempferol glucoside isomer 1	h	0.47	0.25	0.57	0.25	0.38	0.13
		d	0.42	0.15	0.49	0.44	0.55	0.23
19.5	Kaempferol glucoside isomer 2	h	0.79	0.61	0.53	0.10	0.62	0.32
		d	0.50	0.19	0.62	0.24	0.81	0.56
20.6	Kaempferol rhamnoside	h	1.18	1.08	0.33	0.04	0.53	0.49
		d	0.41	0.21	0.40	0.30	0.86	0.72

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### **Methods S3**

LC-MS/MS chromatography was performed on an Agilent 1200 HPLC system. Separation was achieved on an Agilent XDB-C18 column with 50 x 4.6 mm dimensions and a particle size of 1.8  $\mu\text{m}$ . 0.05% formic acid in water (v:v) and acetonitrile were employed as mobile phases A and B respectively. The elution profile was: 0-0.5 min, 90% A in B; 0.5-4 min, 10-90%B in A; 4-4.5 min, 100% B and 4.5-7 min, 90% A in B. The mobile phase flow rate was 1.1 ml min<sup>-1</sup>. The column temperature was maintained at 25°C. The liquid chromatography was coupled to an API 5000 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany) equipped with a Turbospray ion source operated in negative ionization mode. The instrument parameters were optimized by infusion experiments with pure standards of quercetin glucoside, caffeic acid, catechin and naringenin (Sigma-Aldrich). The ionspray voltage was maintained at -4500 eV. The turbo gas temperature was set at 700°C. Nebulizing gas was set at 60 psi, curtain gas at 25 psi, heating gas at 60 psi and collision gas at 7 psi. Multiple reaction monitoring (MRM) was used to monitor analyte parent ion  $\rightarrow$  product ion: m/z 463  $\rightarrow$ 301 (collision energy (CE) -48 V; declustering potential (DP) -60 V) for quercetin glucoside; m/z 178.8  $\rightarrow$ 135.0 (CE -22 V; DP -55 V) for caffeic acid; m/z 305  $\rightarrow$ 125 (CE -28 V; DP -60 V) for galocatechin and m/z 271  $\rightarrow$ 151 (CE -28 V; DP -55 V) for naringenin. Both Q1 and Q3 quadrupoles were maintained at unit resolution. Analyst 1.5 software (Applied Biosystems, Darmstadt, Germany) was used for data acquisition and processing. Relative quantification of individual analytes was achieved by dividing the peak area signal from LC-MS/MS analysis by the fresh weight of each sample.

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**Table S3** Relative quantification (peak area of LC-MS analysis/mg fresh weight) of minor phenolic compounds (pathway intermediates and compounds with lower abundance) in *Acer pseudoplatanus* and *Fagus sylvatica* buds and leaves two hours (h) and two days (d) after simulated browsing. Data based on LC-MS/MS analyses.

Phenolic compound	Time	No treatment		Clipping		Clipping & Saliva	
		Mean	SD	Mean	SD	Mean	SD
<b>Buds</b>							
<i>Acer pseudoplatanus</i>							
Catechin	h	93140.7	55742.3	79165.8	79837.3	154724.7	110459.1
	d	74892.1	56116.1	44489.8	15668.6	73589.6	32004.7
Epicatechin	h	39319.1	28330.2	27118.1	22831.7	80917.6	94572.1
	d	30403.8	21750.2	20371.9	13830.7	33536.9	22307.7
Gallic acid	h	98633.0	26817.9	117295.1	26410.1	103708.7	40314.5
	d	102237.4	36030.4	98833.5	34972.7	144739.4	40827.2
Gallocatechin	h	20225.6	18544.5	8325.6	8047.7	10884.7	3580.9
	d	12615.0	13622.9	5616.8	4760.8	7590.6	4895.7
Epigallocatechin	h	41828.8	38317.3	13959.8	12512.2	30803.2	19367.6
	d	23034.5	22481.3	14410.0	11023.7	15030.0	9469.0
Quercetin glucoside	h	104349.1	45202.6	95002.9	41122.8	216337.9	108911.4
	d	109378.8	36022.9	78090.9	25991.0	121854.6	47920.3
Eriodictyol isomer 1	h	685.8	699.2	536.1	191.0	1319.8	1582.9
	d	469.8	234.8	242.1	94.9	332.1	105.3
Eriodictyol isomer 2	h	1.4	3.6	2.8	2.8	7.6	15.3
	d	0.4	2.1	3.0	2.0	3.7	5.1
Naringenin isomer 1	h	204.3	222.6	151.8	54.1	348.8	399.4
	d	200.0	107.8	82.3	37.5	131.3	51.0
Naringenin isomer 2	h	117.5	57.8	124.1	47.3	205.1	162.1
	d	116.2	36.2	65.5	32.2	87.9	45.2
Caffeic acid	h	225.7	156.5	298.9	185.3	241.7	192.1
	d	417.2	145.2	266.0	120.1	256.4	116.4
<i>Fagus sylvatica</i>							
Catechin	h	353411.7	149544.1	194531.3	63275.1	197858.6	43975.9
	d	241379.2	65102.4	238608.1	55771.5	239195.3	41966.0
Epicatechin	h	215262.2	125905.4	119902.3	52708.8	124324.8	44305.0
	d	101690.6	50078.9	123374.4	83178.6	139095.7	61999.6
Gallic acid	h	2784.7	1659.9	3188.4	1351.4	2604.2	1560.7
	d	4353.4	3342.6	3899.3	2194.5	2250.0	689.7
Gallocatechin	h	256404.9	131230.6	161023.4	74913.9	165337.0	27492.3
	d	339344.2	139812.5	295770.7	124872.0	230209.4	75345.7
Epigallocatechin	h	241247.7	108955.5	147780.5	62046.9	152745.1	49882.2
	d	181766.1	98461.6	169248.4	77911.5	173601.9	70755.4
Quercetin glucoside	h	62632.7	57198.9	30532.5	14099.2	28296.0	17879.8
	d	46559.7	17772.7	43209.4	22322.0	61575.3	33748.8
Eriodictyol isomer 1	h	953.7	992.5	648.2	264.5	546.8	129.5
	d	575.8	298.5	645.0	200.8	653.8	255.2
Eriodictyol isomer 2	h	1042.0	673.6	628.7	274.2	405.9	156.5
	d	973.1	466.3	1068.4	383.7	994.5	525.8
Naringenin isomer 1	h	1356.3	877.7	894.2	461.5	849.4	328.3
	d	750.7	275.5	912.7	279.8	1073.6	396.8
Naringenin isomer 2	h	6988.3	5361.1	3658.2	2211.9	1947.3	372.6
	d	5847.6	3427.3	5944.4	3601.6	5440.1	3442.6

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Caffeic acid	h	2002.7	925.8	939.1	397.8	1168.7	346.6
	d	3279.4	2365.8	2039.9	1344.4	1893.2	1089.7
<b>Leaves</b>							
<i>Acer pseudoplatanus</i>							
Catechin	h	3111.7	1241.8	5019.5	4361.3	2910.5	4375.4
	d	3324.8	2294.1	3257.8	2140.6	4486.8	3045.1
Gallic acid	h	26019.0	9761.1	24596.3	5307.8	31361.0	9763.1
	d	30887.4	10741.7	27079.0	9164.6	32005.4	7880.8
Gallocatechin	h	1049.3	1035.6	6543.7	8895.3	3136.5	6742.2
	d	1723.2	3315.8	843.8	1498.0	2183.1	2310.4
Quercetin glucoside	h	14410.1	16567.8	7072.6	6802.7	16707.4	13635.2
	d	5819.1	6294.1	22010.2	12584.0	15197.7	15261.3
Ferulic acid	h	344.2	234.3	428.4	462.6	480.5	815.1
	d	242.2	336.8	188.5	91.2	306.5	191.0
Epicatechin	h	3126.7	1241.0	5035.7	4351.2	2933.6	4382.7
	d	3352.7	2314.1	2189.0	2150.6	4512.2	3038.6
Epigallocatechin	h	1502.0	1689.4	6487.0	13529.2	6306.3	13854.9
	d	1254.3	2433.6	1351.6	2281.1	1136.7	929.4
<i>Fagus sylvatica</i>							
Catechin	h	60835.5	40471.7	54180.7	16253.4	71084.0	26468.6
	d	64557.0	20460.3	70800.1	27847.0	62292.1	26241.2
Gallic acid	h	3608.0	1244.7	2556.9	714.3	4942.6	2547.2
	d	3130.7	1339.6	2810.9	1014.1	3204.2	1420.7
Gallocatechin	h	100736.8	41631.5	163755.6	25477.0	101928.1	31172.7
	d	79658.1	43444.1	149013.9	134791.9	131063.9	110947.3
Quercetin glucoside	h	24181.4	12848.7	16386.4	9126.8	24503.5	5275.4
	d	15041.8	13881.2	20655.8	13792.6	23373.2	11871.1
Ferulic acid	h	13661.5	7991.1	19543.0	5161.7	15936.6	4747.0
	d	21215.8	2103.4	17985.0	4282.7	20888.1	7182.8
Epicatechin	h	55068.5	47598.5	54368.0	16410.1	71361.1	26700.0
	d	65159.8	20769.7	71354.0	28248.5	62947.0	26734.9
Epigallocatechin	h	94213.0	79285.5	95514.9	86346.1	97068.3	63450.4
	d	97013.2	41007.0	111712.7	98212.2	64414.0	28013.8

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## **Methods S4**

### *Sugar analysis*

Sugars were extracted with 1 mL of 80% methanol solution and the resulting extract was diluted in a ratio of 1:10 (v:v) in water. Sugars in the diluted extracts were directly analysed by LC-MS/MS. Chromatography was performed on an Agilent 1200 HPLC system (Agilent Technologies, Boeblingen, Germany) coupled to an API3200 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany). Separation was achieved on a HILIC-HPLC-column (apHera NH<sub>2</sub> Polymer; 15 x 4,6mm, 5µm, Supelco). Water and acetonitrile were employed as mobile phases A and B respectively. The elution profile was: 0-0.5min, 80%B in A; 0.5-13min, 80-55%B in A; 13-14min, 55-80%% B in A and 14-18min, 80% B in A. The mobile phase flow rate was 1.0 ml/min. The column temperature was maintained at 25°C. The liquid chromatography was coupled to an API 3200 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany) equipped with a Turbospray ion source operated in negative ionization mode. The instrument parameters were optimized by infusion experiments with pure standards (D-(+)-glucose, D-(-)-fructose, sucrose, all Sigma-Aldrich). The ionspray voltage was maintained at at -4500 eV. The turbo gas temperature was set at 600 °C. Nebulizing gas was set at 50psi, curtain gas at 20psi, heating gas at 60psi and collision gas at 5psi. Multiple reaction monitoring (MRM) was used to monitor analyte parent ion → product ion: m/z 178.8 →89.0 (collision energy (CE)-10 V; declustering potential (DP) -25 V) for D-(+)-glucose; m/z 178.8 →89.0 (CE -12 V; DP -25 V) for D-(-)-fructose; m/z 340.9 →59.0 (CE -46 V; DP -55 V) for sucrose. Both Q1 and Q3 quadrupoles were maintained at unit resolution. Analyst 1.5 software (Applied Biosystems, Darmstadt, Germany) was used for data acquisition and processing. Individual sugars in the sample were quantified by external standard curves generated with dilution series of authentic standards.

### *Amino acid analysis*

Amino acids were extracted as for sugar analysis and the resulting extract was diluted in a ratio of 1:10 (v:v) in water containing a <sup>13</sup>C, <sup>15</sup>N labelled amino acid mix (Isotec, Miamisburg, US). Amino acids in the diluted extracts were directly analysed by LC-MS/MS as

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described in Docimo et al. (2012), with the modification, that an API5000 mass spectrometer (Applied Biosystems) was used.

**Docimo T, Reichelt M, Schneider B, Kai M, Kunert G, Gershenzon J, D'Auria J. 2012.** The first step in the biosynthesis of cocaine in *Erythroxylum coca*: the characterization of arginine and ornithine decarboxylases. *Plant Molecular Biology* **78**: 599–615.

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**Table S4** Soluble sugars (mg gFM-1) in *Acer pseudoplatanus* and *Fagus sylvatica* buds and leaves two hours (h) and two days (d) after simulated browsing.

Sugar	Time	<i>Acer pseudoplatanus</i>				<i>Fagus sylvatica</i>							
		No treatment		Clipping		Clipping & Saliva		No treatment		Clipping		Clipping & Saliva	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Buds</b>													
Fructose	h	9.44	3.89	8.92	3.59	7.35	4.39	20.89	9.26	15.97	6.83	14.89	5.93
	d	11.15	5.06	7.47	2.72	3.71	1.60	17.82	5.44	17.46	9.20	20.97	5.30
Glucose	h	1.56	0.71	1.42	0.54	1.17	0.73	3.94	1.02	2.98	0.81	2.74	0.42
	d	2.23	1.21	1.31	0.65	0.65	0.32	3.35	0.64	3.60	1.46	3.59	1.08
Sucrose	h	9.19	1.52	8.06	1.06	7.85	1.73	22.57	8.31	13.52	2.58	14.29	2.86
	d	7.64	2.46	7.13	1.21	6.19	1.71	14.63	3.11	13.00	4.15	15.63	2.35
<b>Leaves</b>													
Fructose	h	0.09	0.07	0.27	0.07	0.23	0.13	0.69	0.40	1.41	0.78	1.03	0.61
	d	0.16	0.09	0.39	0.17	0.32	0.29	1.36	0.90	0.67	0.43	0.70	0.25
Glucose	h	0.03	0.02	0.10	0.04	0.07	0.05	0.57	0.18	0.76	0.12	0.58	0.39
	d	0.06	0.05	0.17	0.16	0.34	0.41	0.92	0.33	0.47	0.32	0.43	0.13
Sucrose	h	0.98	0.55	1.30	0.32	1.23	0.32	4.14	0.41	4.12	0.67	3.26	1.78
	d	1.34	0.26	1.71	0.47	1.36	0.47	4.03	0.67	3.13	1.89	3.72	0.42

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**Table S5** Free amino acids (nmol gFM-1) in *Acer pseudoplatanus* and *Fagus sylvatica* buds and leaves two hours (h) and two days (d) after simulated browsing.

Amino acid	Time	<i>Acer pseudoplatanus</i>				<i>Fagus sylvatica</i>							
		No treatment		Clipping		Clipping & Saliva		No treatment		Clipping		Clipping & Saliva	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Buds</b>													
Alanine	h	6246.0	3952.3	8198.2	4038.4	7180.6	6586.4	640.9	245.1	505.4	308.3	494.2	199.7
	d	12215.5	6377.5	9232.6	3077.9	6217.8	4753.2	1012.9	570.8	860.0	332.4	919.8	337.5
Serine	h	15322.1	6793.1	17388.4	5846.7	13115.0	8974.1	1766.7	542.5	1231.2	294.4	1149.2	201.9
	d	27285.6	6929.2	26848.2	8502.0	19395.4	9234.6	1952.8	631.7	1794.2	567.3	1929.8	528.8
Proline	h	5986.7	2013.7	7832.4	4374.0	6810.8	4754.8	7882.7	2548.6	5013.0	1214.1	5602.8	1354.2
	d	6757.6	4208.7	5593.4	1888.0	5428.6	3327.1	7601.6	3003.5	6406.0	704.9	7585.6	1582.1
Valine	h	2142.7	1046.9	2002.2	1045.2	2104.2	1293.9	256.0	64.4	226.0	98.3	225.0	92.8
	d	2856.4	739.9	3021.0	772.7	1386.8	656.9	334.3	179.2	272.6	50.8	297.2	47.5
Threonine	h	3121.1	1595.8	3472.0	1332.6	3101.2	2147.8	466.0	104.4	393.6	175.8	436.0	173.5
	d	4745.5	1395.4	5043.8	1189.4	2926.4	761.3	452.5	158.9	396.2	74.6	449.2	63.8
Isoleucine	h	1367.3	675.3	1370.2	709.5	1493.4	1168.5	223.3	65.7	174.6	110.3	192.6	60.3
	d	1841.8	614.0	1937.4	332.7	1065.4	320.2	238.9	106.5	229.2	45.4	245.6	63.7
Leucine	h	1624.7	965.1	1456.0	987.6	1853.4	1416.0	86.4	19.7	98.6	80.2	86.6	40.7
	d	1754.3	554.2	2166.0	561.6	1412.4	562.0	113.7	68.4	105.4	23.1	102.2	16.1
Aspartic acid	h	7079.3	9055.2	4969.4	1540.3	7413.2	9487.4	5986.4	9316.2	5916.6	11024.8	3182.4	4857.5
	d	6065.5	1753.7	3888.8	649.6	3424.2	929.5	3396.3	4895.1	8091.6	15100.3	1881.4	587.4
Glutamic acid	h	13596.5	4370.1	14741.6	4139.7	14821.4	7399.8	5456.0	1222.0	4179.6	812.7	4022.8	850.7
	d	18416.4	5261.7	13286.6	1260.8	11810.4	2451.5	7408.5	3178.7	5641.4	1531.3	7070.2	1863.1
Methionine	h	1591.0	661.5	1536.2	720.2	2110.2	1449.1	54.9	11.9	61.4	57.9	36.8	5.5
	d	1328.5	393.0	1366.4	346.2	1722.0	419.7	53.6	22.5	48.6	2.7	54.8	12.0
Histidine	h	1011.5	414.1	1047.2	380.9	1165.6	765.1	596.4	224.2	407.4	97.6	452.6	190.2
	d	2495.0	1101.5	1369.2	407.2	785.6	249.7	1127.2	1278.1	358.4	62.0	540.2	109.4
Phenylalanine	h	939.5	317.4	657.8	266.2	1132.8	755.1	118.0	34.2	95.6	31.7	102.2	28.9
	d	1126.1	481.3	643.4	506.0	386.0	246.2	93.2	20.6	127.6	34.1	145.8	22.9
Arginine	h	1482.2	817.5	2068.8	2680.8	1461.6	755.2	348.5	351.4	334.2	197.8	295.8	270.0
	d	3251.8	1849.7	2337.4	1953.4	495.8	417.2	342.8	342.0	236.2	172.7	371.0	278.8
Tyrosine	h	182.7	63.7	156.0	77.0	168.2	64.7	50.5	10.0	39.8	6.7	48.8	17.3

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Tryptophan	d	343.8	95.5	189.6	48.5	111.8	24.3	60.5	28.0	54.2	7.6	59.4	6.5
	h	95.9	31.4	82.8	23.0	117.4	45.2	53.3	31.7	43.4	14.6	44.8	25.9
Asparagine	d	186.2	88.7	69.4	13.1	66.4	37.3	41.2	11.8	34.4	13.9	51.6	11.1
	h	11294.3	6277.1	12682.4	5252.5	11273.8	5224.8	1216.9	577.7	945.0	477.6	857.4	425.6
Glutamine	d	18721.5	6162.3	13021.6	5470.6	14641.6	9482.2	2187.4	1157.2	2166.0	1306.5	2139.2	1386.6
	h	88971.3	67332.3	98907.0	43686.0	74224.6	61562.9	4829.4	1626.3	4009.8	3070.7	2780.6	1074.7
Lysine	d	63668.1	26068.6	116881.6	31466.6	65081.4	53262.1	13840.8	13834.2	9352.4	8709.9	6706.8	2039.8
	h	179.1	153.3	167.6	182.2	188.6	117.6	21.3	4.7	17.4	8.2	20.0	3.8
	d	221.0	98.4	202.4	126.2	52.0	38.7	29.6	16.6	25.8	13.5	35.0	17.4
<b>Leaves</b>													
Alanine	h	1098.4	228.2	1008.0	110.3	955.8	450.0	310.6	107.9	352.2	42.9	328.6	108.5
	d	782.8	289.0	801.8	144.8	689.2	321.7	312.2	132.7	321.8	97.4	345.8	139.0
Serine	h	536.6	180.3	641.0	207.9	741.2	605.7	196.4	54.0	195.4	69.0	233.0	99.2
	d	878.0	632.4	601.0	338.3	582.4	58.0	417.6	121.9	295.0	90.5	574.4	393.1
Proline	h	54.4	14.9	47.2	9.8	47.2	14.5	32.0	12.7	39.6	8.7	43.8	14.7
	d	42.8	12.1	51.4	15.0	46.2	13.9	41.8	10.8	47.0	28.0	40.4	9.8
Valine	h	36.8	9.1	41.8	12.2	38.4	10.8	20.4	7.6	24.4	6.2	36.0	23.1
	d	27.4	5.2	40.0	17.2	34.2	4.7	29.0	3.2	28.2	8.9	36.6	11.5
Threonine	h	300.0	67.6	271.8	67.4	297.6	112.7	164.2	72.8	189.0	119.2	178.8	96.2
	d	292.6	76.8	299.8	93.6	256.2	28.3	234.2	38.1	194.2	121.6	230.6	81.4
Isoleucine	h	66.8	29.4	55.0	12.0	59.8	25.9	23.2	9.5	26.8	6.6	35.4	22.9
	d	31.4	6.0	65.6	32.6	51.4	5.3	32.8	6.5	31.8	15.1	31.0	7.1
Leucine	h	49.0	18.0	50.4	16.6	38.4	12.9	14.6	5.3	20.0	8.0	26.6	16.6
	d	25.6	5.1	42.8	14.2	36.6	10.3	22.8	7.3	18.6	5.3	19.8	4.6
Aspartic acid	h	607.8	193.7	665.6	241.1	592.6	101.3	617.4	54.4	572.0	57.9	632.0	115.7
	d	742.2	184.0	624.2	157.6	727.0	202.8	534.0	109.0	622.2	215.5	741.6	135.8
Glutamic acid	h	2277.2	433.5	2728.6	1378.3	2465.6	227.0	2938.2	505.2	3037.2	464.6	3025.6	644.9
	d	3308.8	760.1	3014.8	917.8	2935.8	961.2	2997.6	367.9	3041.8	1091.3	3506.8	723.3
Methionine	h	6.2	2.2	8.2	2.3	7.8	2.2	5.4	1.7	4.6	0.9	4.8	1.5
	d	5.2	1.8	7.0	2.2	8.8	3.1	6.4	1.7	6.0	2.1	8.8	1.6
Histidine	h	88.0	21.2	93.6	12.9	103.2	14.4	82.4	13.1	81.4	18.0	81.6	15.2
	d	90.6	5.2	95.0	6.6	98.0	13.3	96.8	15.8	93.2	14.0	94.6	19.5
Phenylalanine	h	75.0	36.1	77.4	26.0	63.8	16.5	31.4	6.4	46.2	10.4	53.8	18.4
	d	34.8	9.0	79.4	34.9	52.6	11.1	32.2	7.0	38.2	7.3	33.6	5.5

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Arginine	h	31.0	6.8	44.6	12.5	32.6	14.2	57.0	30.2	62.4	30.4	73.2	42.2
	d	27.8	10.5	28.8	10.1	31.4	5.4	80.2	31.0	101.0	77.7	86.0	68.4
Tyrosine	h	134.8	144.2	113.0	71.2	90.2	44.6	23.2	7.1	31.2	3.7	31.0	8.3
	d	37.8	25.8	76.2	39.3	52.4	21.2	33.2	14.5	25.2	8.9	30.0	24.6
Tryptophan	h	9.8	6.9	8.8	1.9	9.6	2.6	4.2	1.8	6.8	2.6	5.8	3.1
	d	4.4	0.5	11.0	2.6	8.0	1.2	7.0	4.3	14.4	10.4	6.6	3.6
Asparagine	h	14.2	2.7	21.0	11.0	19.0	10.7	267.6	430.5	531.6	561.5	349.0	620.5
	d	18.0	10.8	17.4	2.5	23.6	10.9	655.6	791.6	586.8	476.0	608.0	308.7
Glutamine	h	679.4	433.8	559.4	128.1	564.2	282.4	666.2	93.8	579.4	55.7	600.6	66.9
	d	753.6	365.6	551.0	158.7	580.6	139.3	988.0	314.3	1300.8	313.3	1448.0	89.1
Lysine	h	33.8	19.3	18.4	5.2	29.8	21.9	8.4	2.1	6.0	1.0	5.8	1.6
	d	20.6	5.5	27.2	6.2	26.2	7.5	8.4	1.1	8.4	3.6	7.2	1.6

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