

Authentication of meat species and net muscle proteins: updating of an old concept

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Supplementary material

Table S1. List of amino acids standards

Form	Amino acid*	Stock solution	Concentration in stock solution (mg/l)
Liquid solution	Ala	I	223
	Arg		435
	Asp		333
	Cystine		300
	Glu		368
	Gly		188
	His		388
	Leu		328
	Ile		328
	Lys		365
	Met		373
	Phe		413
	Pro		288
	Ser		263
	Thr		298
	Tyr		453
	Val		293
Solid	Cys	II	200
	Asn		200
	Gln		200
	Trp		200
	OH-Pro		200
	OH-Lys		200
	3-Met-His		200
	1-Met-His		200
	Cysteic acid		200
	Methionin		200

*L-Alanine (Ala); L-arginine (Arg); L-asparagine (Asn); L-aspartic acid (Asp); L-cysteine (Cys); L-glutamic acid (Glu); L-glutamine (Gln); L-glycine (Gly); L-histidine (His); L-isoleucine (Ile); L-leucine (Leu); L-lysine (Lys); L-methionine (Met); L-phenylalanine (Phe); L-proline (Pro); L-serine (Ser); L-threonine (Thr); L-tyrosine (Tyr); L-valine (Val); L-threonine (Thr); L-tryptophan (Trp); DL-4-hydroxyproline (OH-Pro); cis-L-3-hydroxylysine (OH-Lys); 1-methyl-L-histidine (1-MetHis); 3-methyl-L-histidine (3-MetHis)

Table S2. Individual amino acids working standards concentrations

	Calibration point (mg/l)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Ala	22.3	11.15	5.575	2.230	1.115	0.558	0.223	0.1115	0.05575	0.0223	0.01115	0.00558	0.00223
Arg	43.5	21.75	10.875	4.350	2.175	1.088	0.435	0.2175	0.10875	0.0435	0.02175	0.01088	0.00435
Asp	33.3	16.65	8.325	3.330	1.665	0.833	0.333	0.1665	0.08325	0.0333	0.01665	0.00833	0.00333
Cystine	30.0	15.00	7.500	3.000	1.500	0.750	0.300	0.1500	0.07500	0.0300	0.01500	0.00750	0.00300
Glu	36.8	18.40	9.200	3.680	1.840	0.920	0.368	0.1840	0.09200	0.0368	0.01840	0.00920	0.00368
Gly	18.8	9.40	4.700	1.880	0.940	0.470	0.188	0.0940	0.04700	0.0188	0.00940	0.00470	0.00188
His	38.8	19.40	9.700	3.880	1.940	0.970	0.388	0.1940	0.09700	0.0388	0.01940	0.00970	0.00388
Leu	32.8	16.40	8.200	3.280	1.640	0.820	0.328	0.1640	0.08200	0.0328	0.01640	0.00820	0.00328
Ile	32.8	16.40	8.200	3.280	1.640	0.820	0.328	0.1640	0.08200	0.0328	0.01640	0.00820	0.00328
Lys	36.5	18.25	9.125	3.650	1.825	0.913	0.365	0.1825	0.09125	0.0365	0.01825	0.00913	0.00365
Met	37.3	18.65	9.325	3.730	1.865	0.933	0.373	0.1865	0.09325	0.0373	0.01865	0.00933	0.00373
Phe	41.3	20.65	10.325	4.130	2.065	1.033	0.413	0.2065	0.10325	0.0413	0.02065	0.01033	0.00413
Pro	28.8	14.40	7.200	2.880	1.440	0.720	0.288	0.1440	0.07200	0.0288	0.01440	0.00720	0.00288
Ser	26.3	13.15	6.575	2.630	1.315	0.658	0.263	0.1315	0.06575	0.0263	0.01315	0.00658	0.00263
Thr	29.8	14.90	7.450	2.980	1.490	0.745	0.298	0.1490	0.07450	0.0298	0.01490	0.00745	0.00298
Tyr	45.3	22.65	11.325	4.530	2.265	1.133	0.453	0.2265	0.11325	0.0453	0.02265	0.01133	0.00453
Val	29.3	14.65	7.325	2.930	1.465	0.733	0.293	0.1465	0.07325	0.0293	0.01465	0.00733	0.00293

Cys	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
Asn	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
Gln	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
Trp	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
OH-Pro	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
OH-Lys	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
3-MetHis*	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
1-MetHis*	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.01000	0.00500	0.00200
L-Cysteic acid*	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.0100	0.00500	0.00200
L-Methionine sulfone*	20.0	10.00	5.000	2.000	1.000	0.500	0.200	0.1000	0.05000	0.0200	0.0100	0.00500	0.00200

Table S3. Validation parameters of the U-HPLC-MS method for determination of amino acids in meat

Amino acid	LCL	Linear range	Regression coefficient R^2	RSDr	Hydrolysis
		(mg/g)			(%)
Ala	0.0037	0.0037–0.1858	0.9994	4.5	116
Arg	0.0182	0.0182–0.7250	0.9882	12.3	79
Asp	0.0013	0.0013–1.3875	0.9989	7.9	–
Asn	0.0005	0.0005–0.5000	0.9989	–	–
Cys**	0.0015	0.0015–0.1533	0.9987	14.6	n.d.
Cystine**	0.0032	0.0032–0.0783	0.9882	–	–
Glu	0.0065	0.0065–0.6467	0.9945	6.0	–
Gln	0.0013	0.0013–0.0547	0.9972	–	–
Gly	0.0152	0.0152–0.6083	0.9938	7.9	87
His	0.0007	0.0007–0.1555	0.9929	6.5	117
Leu + Ile	0.0007	0.0007–0.0688	0.9962	5.5	81
Lys	0.0005	0.0005–0.0480	0.988	8.4	85
Met**	0.0043	0.0043–0.2192	0.9953	5.9	86
Phe	0.0012	0.0012–0.1242	0.9922	8.0	93
Pro	0.0008	0.0008–0.1888	0.9971	5.5	114
Ser	0.0012	0.0012–0.0488	0.9989	5.8	103
Thr	0.0003	0.0003–0.3333	0.9956	6.8	82
Tyr	0.0167	0.0167–0.8333	0.996	9.0	101
Val	0.0017	0.0017–0.8333	0.9963	4.5	110
Trp***	0.0017	0.0017–0.3333	0.9961	–	–
OH-Pro	0.0037	0.0037–0.1858	0.9986	13.5	88
OH-Lys	0.0182	0.0182–0.7250	0.9966	14.2	89
3-MetHis	0.0013	0.0013–1.3875	0.9915	9.7	–
1-MetHis	0.0005	0.0005–0.5000	0.9923	8.9	–
Asp + Asn*	–	–	–	7.9	116
Glu + Gln*	–	–	–	6.0	108

*Asparagine and glutamine is converted during hydrolysis to aspartic acid and glutamic acid during hydrolysis, their RSD values and recoveries were determined for the sum of Asp + Asn and Glu + Gln; **Cystine and cysteine are oxidized prior to hydrolysis and are determined as cysteic acid, which is back stoichiometrically converted to cysteine. Methionine is determined as methionine sulfone after oxidation and the methionine content back stoichiometrically converted to cysteine; ***Tryptophan degrades during acid hydrolysis

Table S4. Characteristics of the U-HPLC-MS method for determination of β -alanylhistidine dipeptides in meat

Analyte	LCL	Linear range	Regression	RSDr	Recovery
		(mg/g)	coefficient R^2		(%)
Carnosine	0.008	0.008–0.150	0.9994	7	97
Anserine	0.004	0.004–0.351	0.9989	9	95

Table S5. The values of ratios of particular amino acids to the 3-MetHis in the samples of chicken, pork and beef meat

Amino acid/3 MetHis	Chicken meat		Beef meat		Pork meat	
	range	average	range	average	range	average
Ala	13–22	18	16–20	17	11–21	17
Arg	20–30	27	26–27	27	17–29	23
Asp + Asn	19–28	24	17–30	25	16–29	23
Cystin	2.9–5.4	4.4	3.5–4.9	4.1	2.1–5.1	3.5
Phe	3.2–4.3	3.7	3.8–5.7	4.6	11–16	12
Glu + Gln	54–60	56	48–64	55	34–77	53
Gly	4.5–7.7	6.4	6.6–8.7	7.3	8–16	11
His	12–18	15	11–18	16	11–18	14
OH-Lys	0.30–0.54	0.46	0.77–0.98	0.56	0.35–0.71	0.6
OH-Pro	0.92–2.5	1.5	1.2–1.9	1.6	0.48–1.3	1.0
Leu + Ile	20–28	26	25–27	26	23–33	27
Lys	26–39	35	34–38	36	22–37	31
Met	1.2–1.9	1.7	2.5–4.9	3.5	2.9–5.1	4.2
Pro	2.5–3.5	3.1	3.0–6.9	4.5	6.0–13	9.2
Ser	11–14	14	13–17	14	12–19	15
Thr	13–19	17	18–19	18	16–21	18
Tyr	11–16	14	15–16	15	16–17	15
Val	15–21	19	18–20	19	13–17	15

Table S6. 1-MetHis/3-MetHis ratios

Meat	Ratio	Average
Chicken	7.6–10	8.0
Beef	0.62–0.73	0.66
Pork	0.20–0.29	0.24