

Supplementary material

Signal-Corrected LC–MS/MS Approaches in Amino Acid and Biogenic Amine profiling for Chemometric Characterization of Commercial Dark Chocolates According to Cocoa Content and Manufacturer

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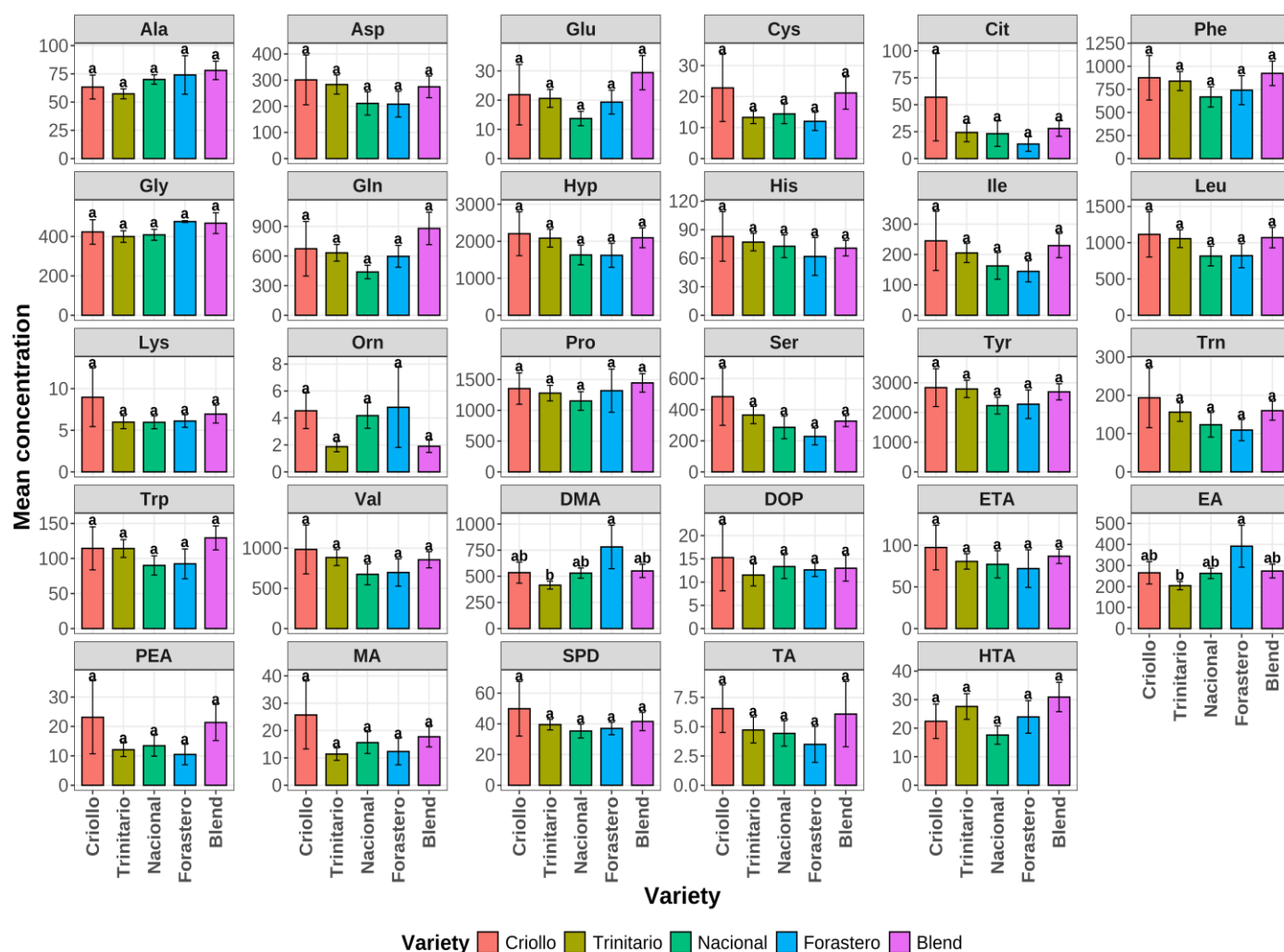


Figure S1. Mean concentrations ($\pm$ SD) of amino acids and amines according to cocoa variety. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$). Abbreviations: Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Cys, cysteine; Cit, citrulline; Phe, phenylalanine; Gly, glycine; Gln, glutamine; Hyp, hydroxyproline; His, histidine; Ile, iso-

leucine; Leu, leucine; Lys, lysine; Orn, ornithine; Pro, proline; Ser, serine; Tyr, tyrosine; Trn, threonine; Trp, tryptophan; Val, valine. DMA, dimethylamine; DOP, dopamine; EA, ethylamine; ETA, ethanolamine; HTA, serotonin; MA, methylamine; PEA, phenylethylamine; SPD, spermidine; TYR, tyramine.

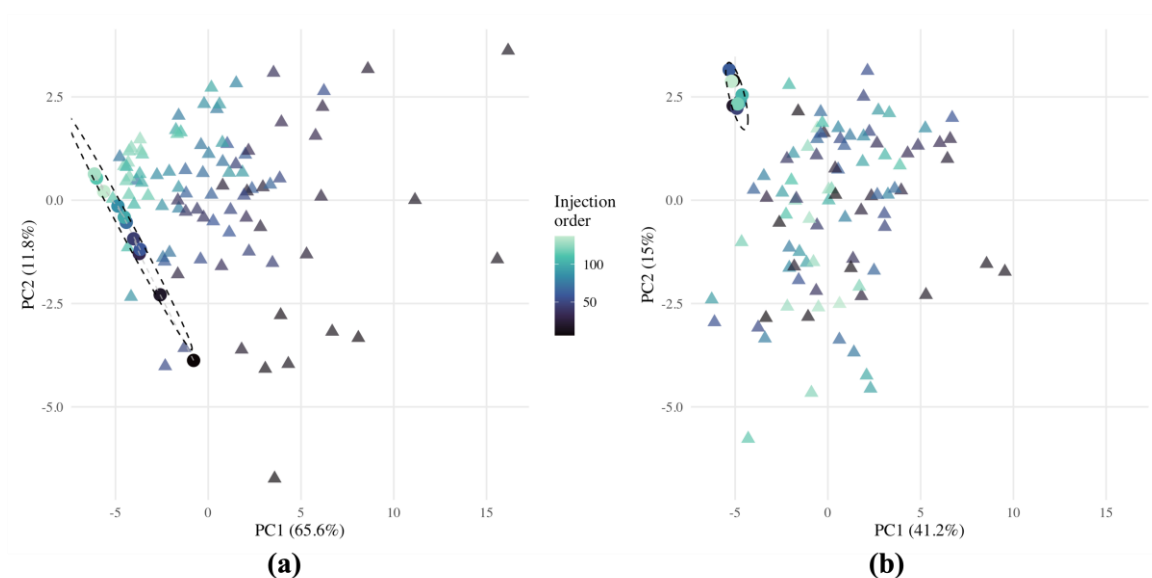


Figure S2. Principal component analysis (PCA) score plots of 91 commercial dark chocolate samples based on amino acid and amine profiles. (a) PCA score plot obtained from the raw data matrix. (b) PCA score plot after data correction. Samples are represented by triangles, whereas QC samples are shown as circles. Symbols are colored according to injection order.

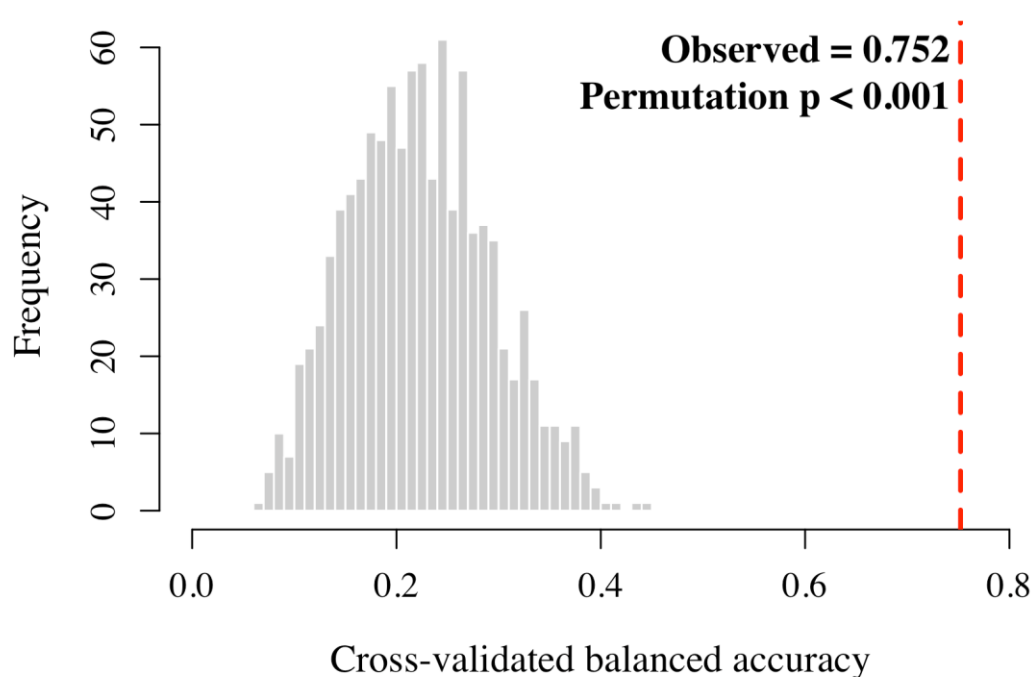


Figure S3. Distribution of cross-validated balanced classification accuracies obtained from 1000 permutation tests. The red vertical line indicates the performance of the optimized PLS-DA model (75.2%). None of the permuted models achieved an equal or higher classification accuracy ($p < 0.001$).

Table S1. List of analytical standards employed in the study, including compound name, abbreviation, and supplier.

Compound	Abbreviation	Supplier
Hydroxyproline	Hyp	Fluka (Buchs, Switzerland)
proline	Pro	
L-glutamic acid	Glu	
L-aspartic acid	Asp	
L-lysine monohydrochloride	Lys	
2-phenylethylamine hydrochloride	PEA	
L-tyrosine	Tyr	Sigma Aldrich (Steinheim, Germany)
L-cysteine	Cys	
Dopamine hydrochloride	DOP	
L-leucine	Leu	
Serotonin hydrochloride	HTA	
L-glutamine	Gln	
L-ornithine monohydrochloride	Orn	Carlo Erba (Milan, Italy)
L-serine	Ser	
L-asparagine	Asn	
Putrescine dihydrochloride	PUT	Alfa Aesar (Massachusetts, USA)
Cadaverine dihydrochloride	CAD	
Dimethylamine hydrochloride	DMA	
Spermine tetrahydrochloride	SPM	
Spermidine trihydrochloride	SPD	

L-Citrulline	Cit	Chemika (St. Gallen, Switzerland)
Ethylamine hydrochloride	EA	
Ethanolamine hydrochloride	ETA	
Methylamine hydrochloride	MA	
Tyramine hydrochloride	TYR	Tokyo Chemical Industry (TCI, Tokyo, Japan)
Hexylamine hydrochloride	HEX	
Butylamine hydrochloride	BUT	
Isopropyl amine hydrochloride	IPA	
Octopamine	OCT	
L-histidine	His	
L-tryptophan	Trp	Thermo Scientific (Waltham, Massachusetts, USA)
L-isoleucine	Ile	
L-valine	Val	
L-glycine	Gly	
L-methionine	Met	
L-alanine	Ala	Merck (Darmstadt, Germany)
Threonine	Trn	
L-phenylalanine	Phe	

Table S2. Characteristics of the 91 commercial dark chocolate samples included in this study, including brand, cocoa content, declared cocoa-bean origin, cocoa variety, certification or quality seal, manufacturing location, and best-before date.

Co de	Brand	Cocoa content (%)	Bean origin (Country (Continent))	Variety	Certification/Seal	Location of manufacture	Best before date
X01	Succhard	70	Dominican Republic (CA)	Trinitario	Organic	Madrid, Spain	2025
X02	Blanxart	80	Ghana (Af)	Not declared	Fairtrade	Barcelona, Spain	2025
X03	Torras	100	Unspecified (SA)	Blend	Organic	Girona, Spain	2025
X04	Chocolate Amatller	85	Ghana (Af)	Not declared	Single origin	Barcelona, Spain	2026
X05	Blanxart	72	Dominican Republic (CA)	Not declared	Organic	Barcelona, Spain	2026
X06	Blanxart	99	Dominican Republic (CA)	Not declared	Organic	Barcelona, Spain	2026
X07	Chocolate Amatller	85	Ecuador (SA)	Not declared	Single origin	Barcelona, Spain	2026
X08	Ethiquable	98	Ecuador (SA)	Nacional	Fairtrade	Italy	2025
X09	Ethiquable	85	Madagascar (Af)	Not declared	Fairtrade	Italy	2026
X10	Ethiquable	72	Haiti (CA)	Not declared	Fairtrade	Italy	2027
X11	Republica del cacao	75	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X12	Republica del cacao	75	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X13	Millesime	74	Guatemala (CA)	Trinitario	Fairtrade	Lliege, Belgium	2025

X14	Blanxart	72	Ghana (Af)	Not declared	Fairtrade	Barcelona, Spain	2025
X15	Succhard	85	Dominican Republic (CA)	Trinitario	Organic	Madrid, Spain	2025
X16	JD Gross	95	Ecuador (SA)	Nacional	Fairtrade	Murcia, Spain	2025
X17	JD Gross	85	Ecuador (SA)	Nacional	Fairtrade	Murcia, Spain	2027
X18	Museu Xocolata	70	Ghana (Af)	Forastero	Not declared	Barcelona, Spain	2025
X19	Museu Xocolata	70	Madagascar (Af)	Trinitario	Single origin	Barcelona, Spain	2025
X20	Anvers Fortuna	100	Peru (SA)	Nacional	Single origin	Tasmania, Australia	2025
X21	Museu Xocolata	70	Tanzania (Af)	Trinitario	Single origin	Barcelona, Spain	2025
X22	Museu Xocolata	70	Dominican Republic (CA)	Trinitario	Organic	Barcelona, Spain	2025
X23	Xocolata Jolonch	72	Venezuela (SA)	Not declared	Not declared	Lleida, Spain	2025
X24	Blanxart	99	Ghana (Af)	Not declared	Fairtrade	Barcelona, Spain	2026
X25	Blanxart	81	Peru (SA)	Not declared	Organic	Barcelona, Spain	2025
X26	Blanxart	91	Congo (Af)	Not declared	Single origin	Barcelona, Spain	2026
X27	Blanxart	78	Brazil (SA)	Not declared	Organic	Barcelona, Spain	2025
X28	Blanxart	87	Nicaragua (CA)	Not declared	Organic	Barcelona, Spain	2025
X29	Blanxart	95	Ecuador (SA)	Trinitario	Single origin	Barcelona, Spain	2025
X30	Aruntam	72	India (A&O)	Not declared	Organic	Milan, Italy	2025
X31	Chocolat Bonnat	75	Sri Lanka (A&O)	Not declared	Not declared	Cedex, France	2025
X32	Kaitxo	70	Philippines (A&O)	Blend	Not declared	Bizkaia, Spain	2025
X33	Utopick	90	Philippines (A&O)	Not declared	Not declared	Valencia, Spain	2025
X34	Utopick	70	Philippines (A&O)	Not declared	Not declared	Valencia, Spain	2026
X35	Chef Kankel	75	Philippines (A&O)	Trinitario	Not declared	Abelda de Iregua, Spain	2025
X36	Blanxart	82	Dominican Republic (CA)	Not declared	Fairtrade	Barcelona, Spain	2025
X37	Blanxart	100	Peru (SA)	Criollo	Organic	Barcelona, Spain	2025
X38	Blanxart	93	Congo (Af)	Not declared	Single origin	Barcelona, Spain	2026
X39	Blanxart	77	Peru (SA)	Criollo	Single origin	Barcelona, Spain	2026
X40	Cacao hunters	64	Colombia (SA)	Not declared	Not declared	Popayan, Colombia	2025
X41	Blanxart	82	Congo (Af)	Forastero	Single origin	Barcelona, Spain	2025
X42	Blanxart	85	Nicaragua (CA)	Not declared	Organic	Barcelona, Spain	2026

X43	Xocolata Jolonch	70	New guinea (A&O)	Not declared	Not declared	Lleida, Spain	2025
X44	Xocolata Jolonch	66	Madagascar (Af)	Not declared	Not declared	Lleida, Spain	2025
X45	Xocolata Jolonch	66	Mexico (CA)	Not declared	Not declared	Lleida, Spain	2025
X46	Sprüngli	65	Venezuela (SA)	Criollo	Not declared	Zurich, Switzerland	2025
X47	Sprüngli	75	Bolivia (SA)	Criollo	Not declared	Zurich, Switzerland	2025
X48	Sprüngli	70	Cuba (CA)	Trinitario	Not declared	Zurich, Switzerland	2025
X49	Sprüngli	60	Ghana (Af)	Forastero	Not declared	Zurich, Switzerland	2025
X50	Republica del cacao	75	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X51	Succhard	70	Dominican Republic (CA)	Trinitario	Fairtrade	Madrid, Spain	2025
X52	JD Gross	56	Unspecified (SA)	Nacional	Fairtrade	Murcia, Spain	2026
X53	Kallari and Yumbos	85	Ecuador (SA)	Nacional	Organic	Quito, Ecuador	2025
X54	Sancao	75	Colombia (SA)	Blend	Not declared	Medellin, Colombia	2025
X55	Paccari	72	Ecuador (SA)	Nacional	Organic	Quito, Ecuador	2025
X56	Dorigenn	70	Colombia (SA)	Nacional	Fairtrade	Tumaco, Colombia	2026
X57	Mindus	77	Ecuador (SA)	Nacional	Organic	Quito, Ecuador	2025
X58	Kallari and Yumbos	75	Ecuador (SA)	Not declared	Organic	Quito, Ecuador	2025
X59	Kallari and Yumbos	70	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X60	Kallari and Yumbos	60	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X61	Chocolate Amatller	72	Nicaragua (CA)	Blend	Organic	Barcelona, Spain	2026
X62	Chocolate Amatller	83	Peru (SA)	Blend	Organic	Barcelona, Spain	2026
X63	Blanxart	76	Brazil (SA)	Trinitario	Single origin	Barcelona, Spain	2026
X64	JD Gross	70	Ecuador (SA)	Nacional	Fairtrade	Murcia, Spain	2026
X65	Xocolata Jolonch	72	Peru (SA)	Not declared	Not declared	Lleida, Spain	2026
X66	Xocolata Jolonch	80	Colombia (SA)	Not declared	Not declared	Lleida, Spain	2025
X67	Chocolate Amatller	74	Madagascar (Af)	Trinitario	Organic	Barcelona, Spain	2026
X68	Chocolate Santander	70	Colombia (SA)	Blend	Single origin	Medellin, Colombia	2026
X69	Kallari and Yumbos	85	Ecuador (SA)	Not declared	Single origin	Quito, Ecuador	2025
X70	Paccari	65	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X71	Paccari	70	Ecuador (SA)	Nacional	Organic	Quito, Ecuador	2025
X72	Republica del cacao	75	Ecuador (SA)	Not declared	Single origin	Quito, Ecuador	2025

X73	Kallari and Yumbos	70	Ecuador (SA)	Not declared	Organic	Quito, Ecuador	2025
X74	Paccari	60	Ecuador (SA)	Nacional	Single origin	Quito, Ecuador	2025
X75	Dorigenn	70	Colombia (SA)	Blend	Fairtrade	Bogota, Colombia	2025
X76	Dorigenn	70	Colombia (SA)	Blend	Fairtrade	Bogota, Colombia	2025
X77	Tierra madre	70	São Tomé and Príncipe (Af)	Not declared	Fairtrade	No reported, Spain	2025
X78	Chocolate Amatller	81	Uganda (Af)	Blend	Organic	Barcelona, Spain	2027
X79	Ethiquable	100	Peru (SA)	Not declared	Fairtrade	Fleurance, France	2026
X80	Millesime	80	Mexico (CA)	Criollo	Fairtrade	Lliege, Belgium	2025
X81	Chocolate Amatller	70	Ghana (Af)	Not declared	Single origin	Barcelona, Spain	2025
X82	Nau Do Cacau	66	São Tomé and Príncipe (Af)	Not declared	Organic	Leiria, Portugal	2025
X83	Puchero	85	Tanzania (Af)	Trinitario	Single origin	Valladolid, Spain	2025
X84	Fjak	80	Tanzania (Af)	Not declared	Single origin	Hardanger, Norway	2026
X85	Moro	75	New guinea (A&O)	Not declared	Not declared	Llerena, Spain	2025
X86	Tibito	70	Colombia (SA)	Not declared	Single origin	Bogota, Colombia	2025
X87	Tibito	70	Colombia (SA)	Not declared	Single origin	Bogota, Colombia	2025
X88	Tibito	70	Colombia (SA)	Not declared	Single origin	Bogota, Colombia	2025
X89	Tibito	70	Colombia (SA)	Not declared	Single origin	Bogota, Colombia	2025
X90	Tibito	70	Colombia (SA)	Not declared	Single origin	Bogota, Colombia	2025
X91	Cacao hunters	70	Colombia (SA)	Not declared	Not declared	Popayan, Colombia	2026

CA, Central America and the Caribbean; SA, South America; Af, Africa; A&O, Asia and Oceania. “Not declared” indicates that the information was not provided on the product label. “Blend” refers to chocolates produced from a mixture of cocoa varieties. Cocoa content corresponds to the percentage declared by the manufacturer.

Table S3. LC-MS/MS multiple reaction monitoring (MRM) acquisition parameters used for the determination of amino acids and amines. The table includes the analytical function of each compound (target analyte, internal standard, or surrogate standard), the number of dansyl groups after derivatization, precursor ion (Q1), product ion (Q3), declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP). MRM transitions and instrumental parameters were adapted from the previously established method described by Navarro-Abril et al. [53].

Compound	Abbreviation	Analytical function	Number of dansyl groups	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
L-Alanine	Ala	Target	1	323	170	100	50	12
L-Aspartic acid	Asp	Target	1	367	170	100	50	12
L-Citrulline	Cit	Target	1	409	170	100	50	12
L-Cysteine	Cys	Target	1	355	170	90	30	10
L-Glutamic acid	Glu	Target	1	381	170	100	50	12
L-Glutamine	Gln	Target	1	380	170	100	50	12
Glycine	Gly	Target	1	309	170	100	50	12
L-Histidine	His	Target	2	622	170	100	50	12
Hydroxyproline	Hyp	Target	1	365	170	100	50	12
L-Isoleucine	Ile	Target	1	365	170	100	50	12
L-Leucine	Leu	Target	1	365	170	100	50	12
L-Lysine monohydrochloride	Lys	Target	2	613	170	100	50	12
L-Methionine	Met	Target	1	383	170	100	50	12
L-Ornithine monohydrochloride	Orn	Target	2	599	170	100	50	12
L-Phenylalanine	Phe	Target	1	399	170	100	50	12
L-Proline	Pro	Target	1	349	170	100	50	12
L-Serine	Ser	Target	1	339	170	100	50	12
Threonine	Trn	Target	1	353	170	100	50	12
L-Tryptophan	Trp	Target	1	438	170	100	50	12
L-Tyrosine	Tyr	Target	2	648	170	100	50	12
L-Valine	Val	Target	1	351	170	100	50	12
Cadaverine dihydrochloride	CAD	Target	2	569	234	100	50	12
Dimethylamine hydrochloride	DMA	Target	1	279	170	100	50	12
Dopamine hydrochloride	DOP	Target	1	387	170	100	50	12
Ethanolamine hydrochloride	ETA	Target	1	295	170	100	50	12
Ethylamine hydrochloride	EA	Target	1	279	170	100	50	12
Methylamine hydrochloride	MA	Target	1	265	170	100	50	12
2-Phenylethylamine hydrochloride	PEA	Target	1	355	170	90	30	10

Compound	Abbrevia tion	Analytical function	Number of dansyl groups	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)
Putrescine dihydrochloride	PUT	Target	2	555	234	120	60	12
Serotonin hydrochloride	HTA	Target	1	410	170	100	50	8
Spermidine trihydrochloride	SPD	Target	3	845	360	40	80	12
Spermine tetrahydrochloride	SPM	Target	4	1135	360	90	50	12
Tyramine hydrochloride	TYR	Target	1	604	234	100	50	12
L-Asparagine	Asn	Internal standard	1	366	170	100	50	12
Isopropylamine hydrochloride	IPA	Internal standard	1	293	170	100	50	12
Octopamine	OCT	Internal standard	1	620	170	66	59	12
Butylamine hydrochloride	BUT	Surrogate standard	1	307	170	125	50	14
Hexylamine hydrochloride	HEX	Surrogate standard	1	335	157	86	39	12

Table S4. Verification of analytical performance for representative analytes. Repeatability was evaluated as the relative standard deviation (RSD, $n = 3$), and linearity was verified over the indicated concentration ranges. These representative compounds were selected to cover different amino acid and amine classes.

Compound	Abbreviation	Repeatability (RSD, %, $n = 3$)	Linear range (mg kg ⁻¹)	R ²
Alanine	Ala	3.5	0.1–5	0.991
Aspartic acid	Asp	5	0.1–35	0.986
Citrulline	Cit	6.4	0.2–25	0.995
Cysteine	Cys	6.8	0.05–10	0.998
Glutamic acid	Glu	6.6	0.1–35	0.992
Glycine	Gly	7.1	0.5–20	0.989
Histidine	His	7.9	0.5–7	0.997
Hydroxyproline	Hyp	7.3	1–35	0.996
Isoleucine	Ile	4.4	0.5–35	0.991
Leucine	Leu	4.7	0.5–27	0.995
Ornithine	Orn	8.6	0.1–5	0.989
Phenylalanine	Phe	5.3	0.2–25	0.995
Proline	Pro	7	0.8–36	0.996
Serine	Ser	8.7	0.5–25	0.986
Tryptophan	Trp	4.4	0.1–10	0.997
Tyrosine	Tyr	4.7	0.8–35	0.992
Valine	Val	4.2	0.2–30	0.996
Dopamine	DOP	7.7	0.1–20	0.985
Ethanolamine	ETA	6.1	0.8–30	0.987
Ethylamine	EA	2.3	0.6–30	0.984
Phenylethylamine	PEA	4.6	0.1–10	0.999

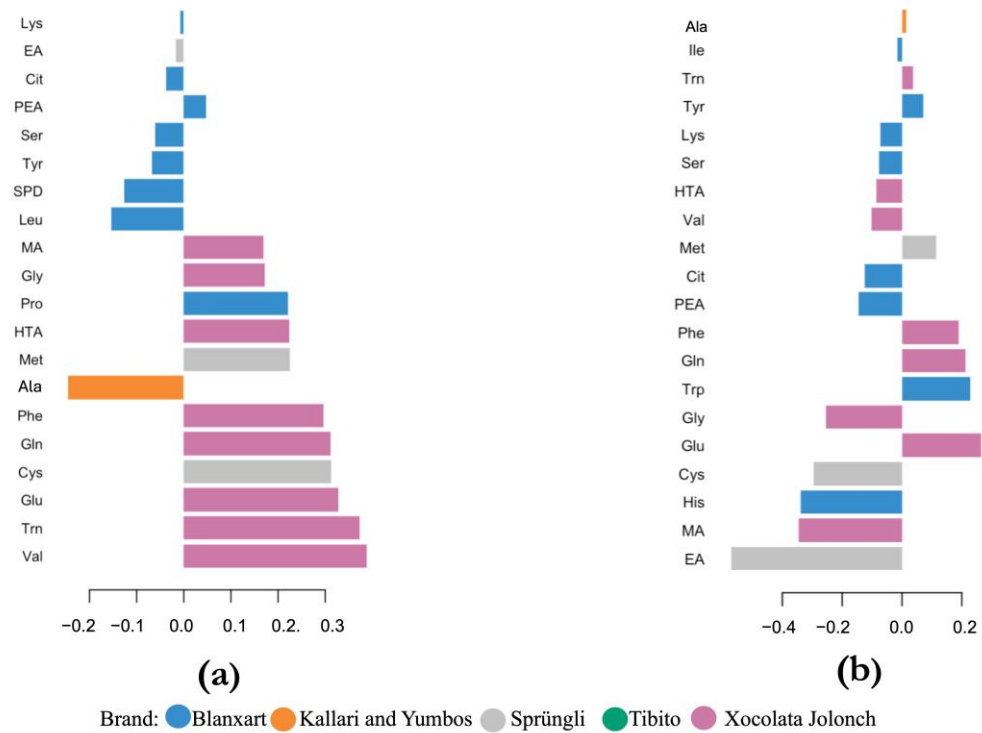


Figure S4. Contribution variables plot of chocolate samples on LV2 (a) and LV3 (b). Colors indicate the brand with the highest median value for each compound. Abbreviations: Lys, lysine; EA, ethylamine; Cit, citrulline; PEA, phenylethylamine; Ser, serine; Tyr, tyrosine; SPD, spermidine; Leu, leucine; MA, methylamine; Gly, glycine; Pro, proline; HTA, hydroxytryptamine (Serotonin); Met, methionine; Ala, alanine; Phe, phenylalanine; Glu, glutamic acid; Cys, cysteine; Gln, glutamine; Trn, threonine; Val, valine; Ile, isoleucine; Trp, tryptophan; His, histidine.