

SUPPLEMENTARY MATERIAL

Discrimination between fresh and frozen-thawed fish involved in food safety and fraud protection

Luca Maria Chiesa^a, Radmila Pavlovic^{a*}, Maria Nobile^a, Federica di Cesare^a, Renato Malandra^b, Davide Pessina, Sara Panseri ^a

^aDepartment of Health, Animal Science and Food Safety, University of Milan, Via Celoria 10, 20133 Milan, Italy

* Corresponding author:

Radmila Pavlovic: Via Celoria, 10 - 20133 Milan (MI) Italy

Tel: +390250317931 Fax: +390250317941

radmila.pavlovic1@unimi.it

Figure S1. Workflow tree from the Compound Discoverer 3.1 software displaying select data processing nodes and the associated workflow connections.

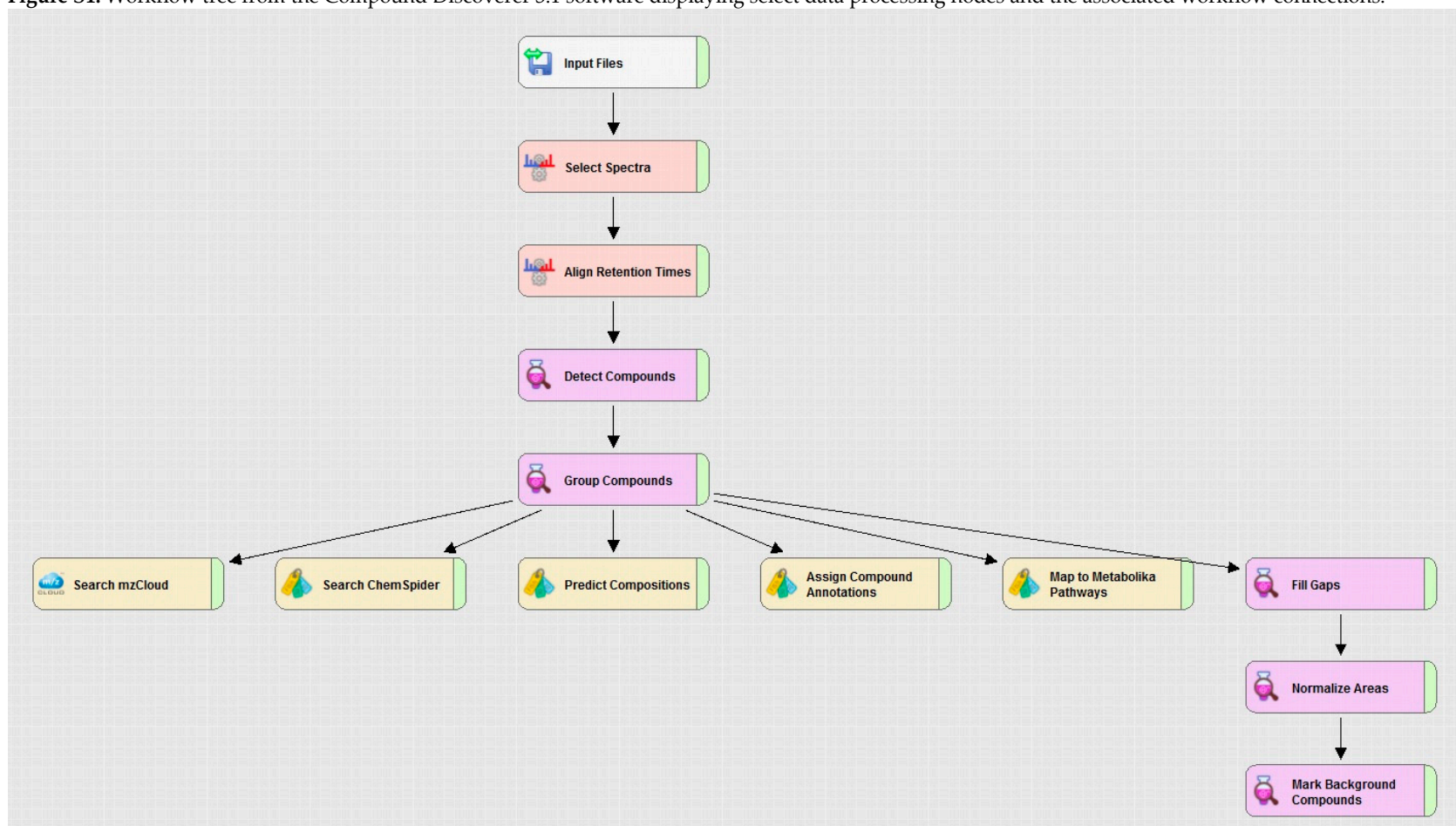


Figure S2. PCA projection on the distribution of fresh and pulled frozen/thawed salmon samples as regards PC-1 with PC-2 when HRMS spectral characterisation of normalised peak area was used.

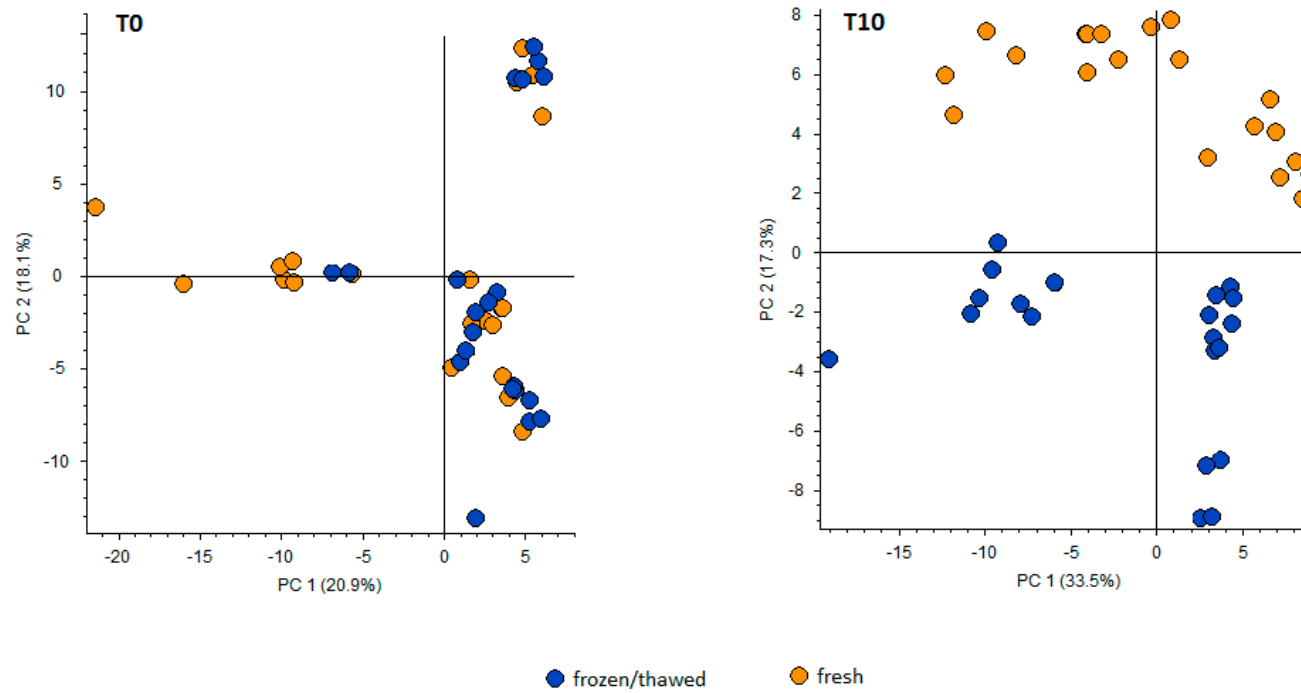
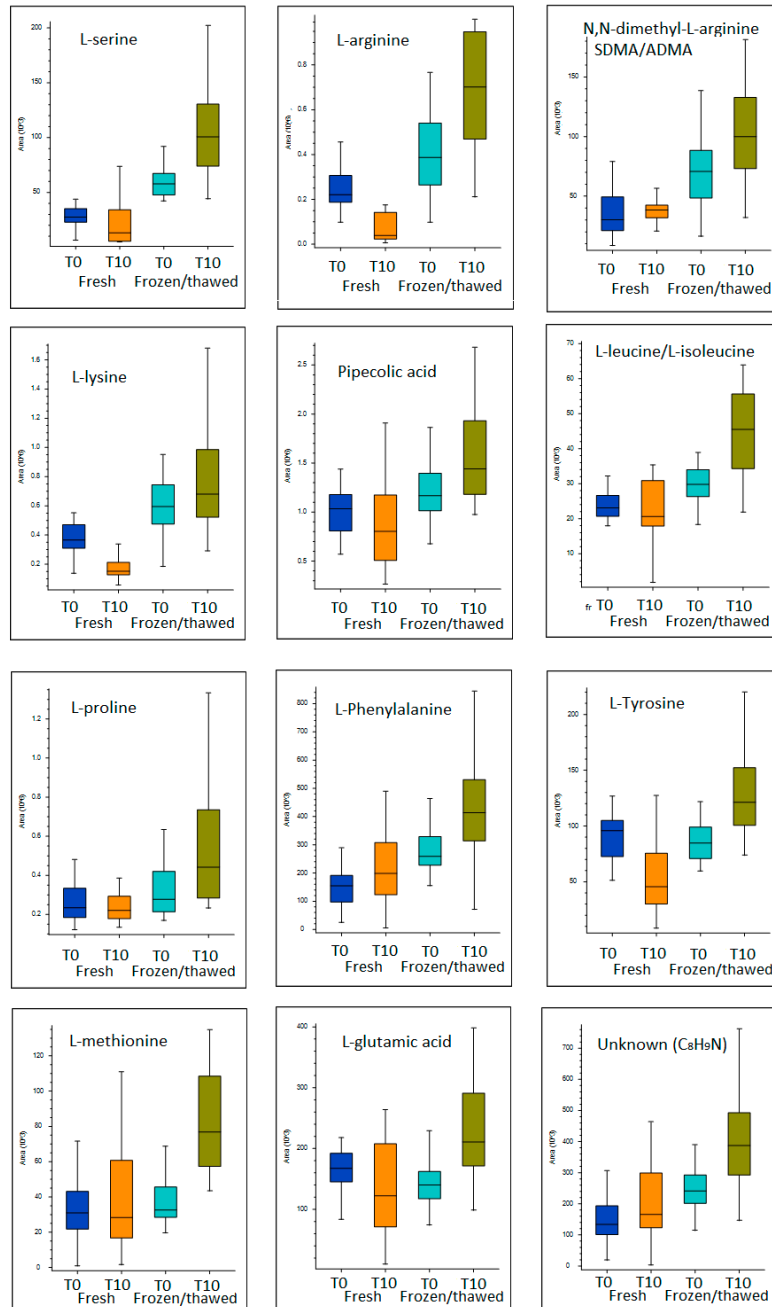


Figure S3. The most important alterations in relative content (normalized peak area) of amino acids and some of their metabolites in fresh salmon samples with corresponding preservation method.



The box diagram representing the interval distribution between 25 and 75%. The horizontal line in the middle of the box represents the median.

Table S1. Identification and semi-quantification of the metabolites detected in salmon samples at T0
(mean \pm SD; $\mu\text{g/g}$ IS equivalents)

Compound	Formula	Exact Mass	Fresh	Frozen/ Thawed			
				- 20 °C		- 35 °C	
				blast chiller	cold room	blast chiller	cold room
<i>Histidine and its metabolites/ derivatives</i>							
<i>Histidine</i>	C6H9N3O2	156.07675	80.1 \pm 41.1	92.6 \pm 41.4	92.7 \pm 51.7	82.4 \pm 42.0	95.4 \pm 55.4
<i>Histamine</i>	C5H9N3	112.08692	0.3 \pm 0.2	0.6 \pm 0.7	0.2 \pm 0.2	0.2 \pm 0.1	0.5 \pm 0.6
<i>3-Methyl-histidine</i>	C7H11N3O2	170.09240	468.1 \pm 105.1	392.3 \pm 81.6	396.8 \pm 82.7	326.0 \pm 109.6	338.2 \pm 74.3
<i>3-Methyl-histamine</i>	C6H11N3	126.10257	34.3 \pm 7.6	28.0 \pm 5.6	29.1 \pm 5.7	32.3 \pm 12.8	34.8 \pm 15.7
<i>Anserine (β-alanyl-3-metil L-histidine)</i>	C10H16N4O3	241.12950	2214.1 \pm 228.8	1197.3 \pm 422.9	1312.3 \pm 553.3	1521.2 \pm 541.3	1638.2 \pm 221.3
<i>Carnosine (β-alanyl-L-histidine)</i>	C9H14N4O3	227.11386	0.7 \pm 0.2	0.4 \pm 0.3	0.5 \pm 0.4	0.2 \pm 0.1	0.2 \pm 0.1
<i>Arginine and its metabolites/ derivatives</i>							
<i>Arginine</i>	C6H14N4O2	175.119	24.3 \pm 9.8	38.3 \pm 6.4	39.7 \pm 16.4	32.3 \pm 7.9	35.8 \pm 17.1
<i>Agmatine</i>	C5H14N4	131.1291	12.0 \pm 20.8	0.3 \pm 0.5	0.4 \pm 0.7	0.3 \pm 0.3	0.1 \pm 0.2
<i>Adma/Sdma (dimethyl-arginine)</i>	C8H18N4O2	203.1503	4.3 \pm 2.8	8.3 \pm 2.1	7.3 \pm 2.9	6.8 \pm 1.8	10.0 \pm 3.9
<i>Nma (metil arginine)</i>	C7H16N4O2	189.1346	0.1 \pm 0.5	0.5 \pm 0.3	1.2 \pm 0.8	0.9 \pm 0.2	1.6 \pm 0.6
<i>Ornithine</i>	C5H12N2O2	133.0972	4.8 \pm 6.5	5.3 \pm 6.3	8.1 \pm 5.0	6.7 \pm 5.5	6.6 \pm 5.6
<i>Carnitine</i>	C7H15NO3	162.1125	48.8 \pm 13.2	42.0 \pm 20.1	40.8 \pm 17.5	25.5 \pm 5.5	36.5 \pm 7.0
<i>N-acyl-carnitine</i>	C9H17NO4	204.1230	56.1 \pm 23.0	43.2 \pm 12.4	52.4 \pm 14.4	48.5 \pm 19.6	53.9 \pm 16.7
<i>Spermine</i>	C12H28N4O	245.2336	24.9 \pm 6.9	18.0 \pm 5.9	26.5 \pm 9.7	15.8 \pm 2.9	28.0 \pm 10.1
<i>Spermidine</i>	C7H19N3	146.1652	36.9 \pm 16.6	34.8 \pm 5.3	27.3 \pm 11.9	30.5 \pm 12.4	32.8 \pm 16.7
<i>Creatinine</i>	C4H7N3O	114.0661	31.9 \pm 8.8	28.8 \pm 1.2	26.7 \pm 20.0	26.1 \pm 6.9	27.2 \pm 2.8
<i>Creatine</i>	C4H4N3O2	132.0767	3199.5 \pm 598.6	2896.9 \pm 521.2	3230.7 \pm 940.2	2171.2 \pm 677.2	2907.1 \pm 189.6
<i>Lysine and its metabolites/ derivatives</i>							
<i>Lysine</i>	C6H14N2O2	147.1128	35.5 \pm 5.2	57.1 \pm 23.8	52.3 \pm 17.9	51.6 \pm 13.4	63.2 \pm 12.3
<i>Cadaverine</i>	C5H14N2	103.1230	nd*	nd	nd	nd	nd
<i>D-Pipecolic acid</i>	C6H11NO2	130.0863	2.7 \pm 0.7	3.7 \pm 2.7	5.2 \pm 3.8	13.8 \pm 8.1	25.8 \pm 1.1
<i>1-Piperideine</i>	C5H9N	84.0808	7.5 \pm 2.1	9.6 \pm 4.3	10.2 \pm 3.4	10.3 \pm 2.1	12.2 \pm 1.9
<i>Other amino acids and their metabolites/derivatives</i>							
<i>Proline</i>	C5H9NO2	116.0706	42.5 \pm 8.4	29.2 \pm 13.9	48.1 \pm 22.0	27.9 \pm 9.1	24.0 \pm 11.3
<i>Leucine/isoleucine</i>	C6H13NO2	132.1019	27.6 \pm 9.3	29.2 \pm 13.9	45.1 \pm 18.0	27.9 \pm 9.1	34.0 \pm 21.3
<i>Alanine</i>	C3H7NO2	90.0550	72.5 \pm 12.4	65.9 \pm 9.9	76.1 \pm 19.8	64.5 \pm 15.9	67.7 \pm 6.4
<i>Serine</i>	C3H7NO3	106.0499	2.0 \pm 0.9	3.2 \pm 1.1	3.0 \pm 0.7	2.5 \pm 0.3	3.4 \pm 1.0
<i>Valine</i>	C5H11NO2	118.0863	149.7 \pm 76.5	130.7 \pm 20.1	201.7 \pm 163.2	100.7 \pm 24.9	161.2 \pm 51.4
<i>Glutamic acid</i>	C5H9NO4	148.0604	14.4 \pm 4.2	11.2 \pm 2.2	11.0 \pm 2.4	9.8 \pm 3.6	8.9 \pm 1.6
<i>Pyroglutamic acid</i>	C5H7NO3	130.0499	4.81 \pm 1.62	8.01 \pm 7.85	14.06 \pm 16.25	3.92 \pm 1.16	4.0 \pm 1.3
<i>Methionine</i>	C5H11NO2S	150.0583	5.4 \pm 3.1	4.5 \pm 0.8	6.3 \pm 2.6	5.0 \pm 2.0	5.2 \pm 2.4
<i>Phenylalanine</i>	C9H11NO2	166.0863	15.7 \pm 7.3	25.9 \pm 4.2	25.6 \pm 4.0	22.9 \pm 1.5	28.2 \pm 8.4
<i>Tyrosine</i>	C9H11NO3	182.0812	12.2 \pm 5.0	10.8 \pm 1.9	12.2 \pm 4.3	10.4 \pm 3.9	11.4 \pm 1.6

<i>Tryptophan</i>	<i>C11H12N2O2</i>	205.0972	2.2 ± 0.6	2.0 ± 0.3	1.8 ± 0.3	1.7 ± 0.1	1.9 ± 0.8
<i>Cysteine</i>	<i>C6H12N2O4S2</i>	241.0311	3.0 ± 0.8	2.2 ± 0.8	2.6 ± 1.0	1.9 ± 0.8	2.1 ± 0.5
<i>Taurine</i>	<i>C2H7NO3</i>	126.0219	17.7 ± 7.1	25.9 ± 4.2	38.6 ± 4.0	24.9 ± 2.5	38.2 ± 0.4
<i>O-phosphate amino derivate</i>							
<i>N-methyl-ethanolamine phosphate</i>	<i>C3H10NO4P</i>	156.0420	27.78 ± 13.01	32.4 ± 22.2	35.65 ± 20.92	16.58 ± 3.60	30.4 ± 3.1
<i>Amino oxides</i>							
<i>Trimethylamine N-oxide</i>	<i>C3H9NO</i>	76.0757	581.24 ± 217.67	508.25 ± 201.10	543.99 ± 226.2	535.84 ± 119.04	664.5 ± 195.25
<i>Purines base</i>							
<i>Hypoxanthine</i>	<i>C5H4N4O</i>	137.0458	264.6 ± 51.6	222.9 ± 57.2	175.2 ± 112.3	211.2 ± 51.4	239.2 ± 0.5
<i>Xanthine</i>	<i>C5H4N4O2</i>	153.0407	0.3 ± 0.1	0.2 ± 0.07	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1

*nd-not detected

Table S2. Identification and semi-quantification of the metabolites detected in salmon samples at T10 (mean \pm SD; $\mu\text{g/g}$ IS equivalents)

Compound	Formula	Exact Mass	Fresh	Frozen/ Thawed			
				- 20 °C		- 35 °C	
				blast chiller	cold room	blast chiller	cold room
<i>Histidine and its metabolites/ derivatives</i>							
Histidine	C5H9N3O2	156.07675	94.1 \pm 41.1	124.7 \pm 37.2	124.2 \pm 43.2	130.6 \pm 32.6	158.6 \pm 64.6
Histamine	C6H9N3	112.08692	38.2 \pm 13.3	1.6 \pm 1.55	2.8 \pm 1.8	1.9 \pm 2.9	2.5 \pm 2.6
3-Methyl-histidine	C7H11N3O2	170.09240	551.2 \pm 222.7	566.9 \pm 96.8	547.7 \pm 190.2	476.9 \pm 145.9	515.3 \pm 189.9
3-Methyl-histamine	C6H11N3	126.10257	42.2 \pm 10.2	40.0 \pm 6.8	40.0 \pm 11.9	35.3 \pm 11.3	36.3 \pm 11.2
Anserine (β -alanyl-3-metil L-histidine)	C10H16N4O3	241.12950	2362.2 \pm 668.3	2210.9 \pm 646.1	2297.9 \pm 622.1	2122.4 \pm 651.9	1910.4 \pm 493.4
Carnosine (β -alanyl-L-histidine)	C9H14N4O3	227.11386	0.8 \pm 0.7	1.0 \pm 0.7	1.6 \pm 1.1	0.6 \pm 0.5	0.6 \pm 0.4
<i>Arginine and its metabolites/ derivatives</i>							
Arginine	C6H14N4O2	175.119	11.4 \pm 12.3	78.5 \pm 9.2	113.4.7 \pm 70.3	56.3 \pm 17.9	48.8 \pm 31.1
Agmatine	C5H14N4	131.1291	124.8 \pm 82.5	11.6 \pm 16.1	13.1 \pm 10.2	19.2 \pm 0.3	20.2 \pm 2.2
Adma/Sdma (dimethyl-arginine)	C8H18N4O2	203.1503	5.2 \pm 3.1	11.1 \pm 2.0	19.6 \pm 2.9	19.8 \pm 7.7	20.0 \pm 13.9
Nma (methyl-arginine)	C7H16N4O2	189.1346	0.7 \pm 0.5	8.5 \pm 11.4	1.8 \pm 0.2	0.6 \pm 0.3	1.7 \pm 1.6
Ornithine	C5H12N2O2	133.0972	8.9 \pm 6.8	8.2 \pm 2.0	12.9 \pm 10.0	1.7 \pm 0.8	6.1 \pm 5.5
Carnitine	C7H15NO3	162.1125	76.8 \pm 41.7	61.5 \pm 12.3	60.2 \pm 12.2	46.8 \pm 17.7	49.8 \pm 22.4
N-acyl-carnitine	C9H17NO4	204.1230	34.8 \pm 12.5	66.4 \pm 13.2	55.1 \pm 27.2	53.3 \pm 11.2	62.2 \pm 21.9
Spermine	C12H28N4O	245.2336	38.6 \pm 10.4	36.0 \pm 1.9	33.5 \pm 1.7	32.8 \pm 12.9	28.0 \pm 10.1
Spermidine	C7H19N3	146.1652	37.9 \pm 267	44.8 \pm 10.3	17.3 \pm 10.9	31.5 \pm 7.4	29.8 \pm 15.7
Creatinine	C4H7N3O	114.0661	38.4 \pm 8.2	34.8 \pm 1.3	33.6 \pm 3.8	30.1 \pm 7.0	48.2 \pm 22.8
Creatine	C4H4N3O2	132.0767	3286.2 \pm 1125.2	3269.2.9 \pm 328.2	3588.7 \pm 576.2	3371.2 \pm 642.2	3319.1 \pm 914.6
<i>Lysine and its metabolites/ derivatives</i>							
Lysine	C6H14N2O2	147.1128	22.8 \pm 12.1	59.1 \pm 26.8	93.3 \pm 53.9	62.0 \pm 26+4	97.4 \pm 24.3
Cadaverine	C5H14N2	103.1230	27.8 \pm 9.1	nd*	nd	nd	nd
D-Pipecolic acid	C6H11NO2	130.0863	12.7 \pm 12.7	33.4 \pm 2.7	35.2 \pm 14.8	23.8 \pm 11.1	45.8 \pm 1 1.1
1-Piperidine	C5H9N	84.0808	5.7 \pm 2.75	16.5 \pm 4.70	19.45 \pm 9.97	13.33 \pm 5.86	20.5 \pm 11.4
<i>Other amino acids and their metabolites/derivatives</i>							
Alanine	C3H7NO2	90.0550	72.5 \pm 12.4	65.9 \pm 9.9	76.1 \pm 19.8	64.5 \pm 15.9	67.7 \pm 6.4
Serine	C3H7NO3	106.0499	1.6 \pm 1.3	7.2 \pm 1.1	7.5 \pm 3.7	4.9 \pm 2.3	7.4 \pm 1.0
Valine	C5H11NO2	118.0863	180.1 \pm 99.4	180.7 \pm 7.7	372.3 \pm 21.7	200.6 \pm 106.9	310.2 \pm 106.4
Glutamic acid	C5H9NO4	148.0604	11.0 \pm 7.1	19.2 \pm 2.5	22.0 \pm 12.5	18.2 \pm 7.4	22.9 \pm 15.6
Pyroglutamic acid	C5H7NO3	130.0499	4.5 \pm 1.5	9.9 \pm 2.1	10.8 \pm 4.2	8.2 \pm 3.1	11.8 \pm 5.2
Methionine	C5H11NO2S	150.0583	5.4 \pm 3.1	4.5 \pm 0.8	6.3 \pm 2.6	5.0 \pm 2.0	5.2 \pm 2.4
Phenylalanine	C9H11NO2	166.0863	3.6 \pm 1.5	10.7 \pm 1.8	12.1 \pm 8.6	10.0 \pm 3.6	18.2 \pm 12.4
Tyrosine	C9H11NO3	182.0812	23.6 \pm 12.8	49.9 \pm 7.2	57.6 \pm 19.0	36.9 \pm 13.5	53.2 \pm 32.4
Tryptophan	C11H12N2O2	205.0972	2.8 \pm 1.2	4.0 \pm 0.4	4.8 \pm 2.5	3.0 \pm 0.1	5.3 \pm 5.1
Cysteine	C6H12N2O4S2	241.0311	3.8 \pm 1.4	3.4 \pm 1.7	3.2 \pm 1.3	3.1 \pm 1.7	2.2 \pm 0.5
Taurine	C2H7NO3	126.0219	8.1 \pm 2.1	15.9 \pm 4.1	13.6 \pm 24.0	14.8 \pm 1.5	18.2 \pm 1.4

<i>O-phosphate amino derivate</i>							
<i>N-methyl-ethanolamine phosphate</i>	<chem>C3H10NO4P</chem>	156.0420	23.8 ± 12.9	32.67 ± 5.8	54.1 ± 2.5	39.82 ± 18.2	47.2 ± 12.8
<i>Amino oxides</i>							
<i>Trimethylamine N-oxide</i>	<chem>C3H9NO</chem>	76.0757	264.6 ± 26.25	627.2 ± 123.2	590.0 ± 37.12	438.1 ± 123.2	541.0 ± 54.1
<i>Purines base</i>							
<i>Hypoxanthine</i>	<chem>C5H4N4O</chem>	137.0458	400.9 ± 104.0	365.2 ± 53.2	350.6 ± 90.3	290.2 ± 95.4	317.2 ± 134.5
<i>Xanthine</i>	<chem>C5H4N4O2</chem>	153.0407	2.1 ± 1.8	0.5 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1

*nd-not detected

Table S3. Identification and semi-quantification of the metabolites detected in the Bullet tuna sample (mean \pm SD; $\mu\text{g/g}$ IS equivalents)

	Formula	Exact Mass	Fresh	Frozen/ Thawed		
				15days	30days	90days
<i>Histidine and its metabolites/ derivatives</i>						
Histidine	C5H9N3O2	156.07675	8294.1 \pm 278.1	1024.8 \pm 125.2	1024.2 \pm 43.2	1300.6 \pm 32.6
Histamine	C6H9N3	112.08692	nd	nd	0.9 \pm 0.5	nd
3-Methyl-histidine	C7H11N3O2	170.09240	1022.2 \pm 227.7	22.9 \pm 16.8	57.7 \pm 10.2	86.9 \pm 45.8
3-Methyl-histamine	C6H11N3	126.10257	22.7 \pm 12.7	33.4 \pm 2.7	35.2 \pm 14.8	23.8 \pm 11.1
Anserine (β -alanyl-3-metil L-histidine)	C10H16N4O3	241.12950	82.1 \pm 8.3	6.9 \pm 1.1	5.9 \pm 32.1	4.4 \pm 2.9
Carnosine (β -alanyl-l-histidine)	C9H14N4O3	227.11386	9.4 \pm 1.2	nd	nd	nd
<i>Arginine and its metabolites/ derivatives</i>						
Arginine	C6H14N4O2	175.119	22.4 \pm 12.3	58.5 \pm 19.2	43.7 \pm 20.1	66.3 \pm 7.9
Agmatine	C5H14N4	131.1291	2.8 \pm 0.3	nd	nd	nd
Adma/Sdma -dimethyl-arginine)	C8H18N4O2	203.1503	0.4 \pm 0.1	nd	nd	nd
Nma (methyl- arginine)	C7H16N4O2	189.1346	0.9 \pm 0.3	nd	nd	nd
Ornithine	C5H12N2O2	133.0972	4.4 \pm 42.3	nd	nd	nd
Carnitine	C7H15NO3	162.1125	146.8 \pm 21.7	162.5 \pm 48.3	160.2 \pm 22.2	126.8 \pm 27.7
N-acyl-carnitine	C9H17NO4	204.1230	44.8 \pm 12.5	66.4 \pm 33.2	58.1 \pm 27.2	63.3 \pm 21.2
Spermine	C12H28N4O	245.2336	64.2 \pm 70.4	62.0 \pm 11.9	53.5 \pm 10.7	42.8 \pm 22.9
Spermidine	C7H19N3	146.1652	27.9 \pm 16.7	24.8 \pm 19.3	37.3 \pm 15.9	41.5 \pm 17.4
Creatinine	C4H7N3O	114.0661	80.4 \pm 40.2	14.7 \pm 9.7	7.1 \pm 45.9	4.5 \pm 7.4
Creatine	C4H4N3O2	132.0767	6258.2 \pm 925.2	3125.9 \pm 415.2	3222.7 \pm 586.2	3371.2 \pm 1652.2
<i>Lysine and its metabolites/ derivatives</i>						
Lysine	C6H14N2O2	147.1128	122.8 \pm 42.1	109.1 \pm 16.8	83.3 \pm 13.9	72.0 \pm 46+4
Cadaverine	C5H14N2	103.1230	nd	nd	nd	nd
<i>Other amino acids and their metabolites/derivatives</i>						
Proline	C5H9NO2	116.0706	102.2 \pm 2.3	30.4 \pm 11.7	30.2 \pm 15.3	30.1 \pm 10.7
Alanine	C3H7NO2	90.0550	122.5 \pm 12.4	65.9 \pm 9.9	66.1 \pm 18.8	44.5 \pm 11.9
Valine	C5H11NO2	118.0863	2521.1 \pm 129.4	100.7 \pm 7.7	72.0 \pm 8.7	125.6 \pm 84.9
Glutamic acid	C5H9NO4	148.0604	41.0 \pm 9.1	8.2 \pm 3.5	12.0 \pm 2.5	7.2 \pm 2.4
Pyroglutamic acid	C5H7NO3	130.0499	28.5 \pm 12.5	8.9 \pm 1.1	7.8 \pm 1.2	1.2 \pm 3.1
Methionine	C5H11NO2S	150.0583	15.4 \pm 8.1	4.5 \pm 0.8	5.3 \pm 2.6	1.0 \pm 0.8
Phenylalanine	C9H11NO2	166.0863	96.6 \pm 14.5	20.7 \pm 2.8	22.1 \pm 8.6	10.0 \pm 3.6
Tyrosine	C9H11NO3	182.0812	1.8 \pm 1.4	nd	nd	nd
Tryptophan	C11H12N2O2	205.0972	42.8 \pm 21.2	2.0 \pm 0.5	1.8 \pm 0.5	5.1 \pm 0.8
Cysteine	C6H12N2O4S2	241.0311	13.8 \pm 11.4	4.4 \pm 1.8	3.1 \pm 0.3	2.1 \pm 0.7
Taurine	C2H7NO3	126.0219	18.1 \pm 3.1	0.8 \pm 0.1	3.6 \pm 2.0	2.8 \pm 0.5
<i>peptides</i>						
γ -glutamyl-S-methylcysteinyl- β -alanine	C12H21N3O6S	336.1225	109.2 \pm 12.8	12.4 \pm 4.7	10.7 \pm 5.3	7.1 \pm 4.7
Glutathione (Reduced)	C10H17N3O6S	308.0908	2.8 \pm 1.2	nd	nd	nd
<i>O-phosphate amino derivate</i>						

<i>N</i> -methyl-ethanolamine phosphate	C ₃ H ₁₀ NO ₄ P	156.0420	42.8 ± 22.5	100.6 ± 10.2	205.1 ± 98.5	350.8 ± 10.2
<i>L</i> -α-Glycerol-phosphoryl-choline	C ₃ H ₁₀ NO ₄ P	258.1099	7.8 ± 7.6	52.2 ± 12.2	42.1 ± 12.8	20.7 ± 14.9
<i>Amino oxides</i>						
Trimethylamine <i>N</i> -oxide	C ₃ H ₉ NO	76.0757	351.6 ± 226.25	225.2 ± 175.2	125.0 ± 37.12	447.1 ± 151.2
<i>Purines base</i>						
Hypoxanthine	C ₅ H ₄ N ₄ O	137.0458	1320.9 ± 221.0	105.2 ± 13.2	125.6 ± 40.3	190.4 ± 85.4
Inosinic acid	C ₁₀ H ₁₃ N ₄ O ₈ P	153.0407	8.1 ± 2.2	1.1 ± 2.2	0.2 ± 0.1	2.8 ± 1.2

*nd-not detected