Saccharomyces cerevisiae and *Torulaspora delbrueckii* Intra- and Extra-Cellular Aromatic Amino Acids Metabolism

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Abbreviations Used

TOL: tryptophol; NIC: nicotinamide; TYR-EE: tyrosine ethyl ester; TYL: tyrosol; IAA-EE: indole acetic acid methyl ester; PHE: phenylalanine; OH-Ph-Py: OH-phenyl-pyruvic acid; N-TRP-EE: N-acetyl-tryptophan ethyl ester; Ph-Py: phenyl-pyruvic acid; KYNA: kynurenic acid; N-TYR-EE: N-acetyl-tyrosine ethyl ester; ANT: anthranilic acid; CH3O-IAA: methoxy-indole acetic acid; IPY: indole pyruvic acid; IAA: indole acetic acid; ICA: indole carboxaldehyde; 2AA: 2-aminoacetophenone; E-ICA ethyl indole carboxaldehyde; ILA: indole lactic acid; TRP-EE: tryptophan ethyl ester; TOL-SO3H: sulfonated tryptophol; TRP-ME: tryptophan methyl ester; TRP: tryptophan; KYN: kynurenine; IBA: indole butyric acid; MEL: melatonin.

Supporting Information Table S1. Standards

Sigma-Aldrich SL Madrid, Spain	**Sigma-Aldrich Quimica SL Milan, Italy**	
tryptamine (TRYPT)	1-cyclohexene-1-carboxylic acid	phenyl pyruvic acid (Ph-Py)
3-Indole acetic acid (IAA)	2-amino acetophenone (2AA)	picolinic acid
3-indole butyric acid (IBA)	2-hydroxy-phenyl acetic acid	shikimic acid
3-indole pyruvic acid (IPy)	3-ethyl-indole carboxylic acid (E-I Ca)	tryptophan methyl ester (TRP ME)
4-hydroxy-phenyl acetic acid (OH-Ph-AA)	3-hydroxy kynurenine (OH-KYN)	tyramine (TYRA)
5-hydroxy-L-tryptophan (OH-TRP)	3-hydroxy-anthranilic acid (OH-ANT)	tyrosine (TYR)
5-Methoxytryptamine (5MOT)	3-indole acetic acid ethyl ester (IAA-EE)	tyrosine methyl ester (TYRME)
5-Metoxy tryptophan (CH_3O-TRP)	3-indole lactic acid (ILA)	formic acid (LC-MS grade)
5-metoxy-3-indole acetic acid (CH_3O-IAA)	3-indole propionic acid (IPA)	methanol (LC-MS grade)
5-Metoxytryptophol (5-HTOL	3-methoxy tyramine	
6-Hydroxymelatonin (OH-MEL)	3-methyl-indole (CH_3-IND)	
DL-kynurenine (KYN)	3-nitrotyrosine(IS)	
DL-tryptophan methyl ester (TRP-ME)	3,4-dihydroxy-3-methoxyphenyl propionic acid	
hydroxy indole -3-acetic acid (5H-IAA)	3(2,4-dihydroxy) phenyl propionic acid	
kynurenic acid (KYNA)	4-hydroxy-phenyl pyruvic acid (OH-Ph-Py)	
L-Tyrosine (TYR)	5-methoxy tryptophol	
L-tyrosine methyl ester (TYR-ME)	6-benzyloxy-6-methoxy indole	
melatonin (MEL)	abscisic acid	
N-acetyl serotonin (N-SER)	anthranilic acid (ANT)	
N-acetyl tryptophan ethyl ester (N-TRP-EE)	dopamine (DOPA)	
N-acetyl-L-tyrosine ethyl ester (N-TYR-EE)	ethyl anthranilate	
phenyl acetic acid (Ph-AA)	indole (IND)	
serotonine (SERO)	indole acetamide	
tryptophan (TRP)	indole acetic acid methyl ester (IAAME)	
tryptophan ethyl ester (TRP-EE)	indole carbinol (I3C)	
tryptophol (TOL)	indole carboxaldehyde (ICA)	
tyrosine ethyl ester (TYR-EE)	indole-2-carboxylic acid	
tyrosol (TYL)	indoxyl sulphate	
	methyl-indole acetic acid (M-IAA)	
Cymit Quimica S.L., Barcelona, Spain.	nicotinamide (NIC)	
N-acetyl-5-methoxy kynureanine hydrochloride (AMK)	nicotinic acid	
sulfatoxy melatonin (6-aMTs)	phenyl alanine (PHE)	
N-γ-acetyl-N-2-formyl-5-methoxy kynureamine (AFMK)	phenyl lactic acid (Ph-LA)	

Supporting Information Table S2. Composition of synthetic must

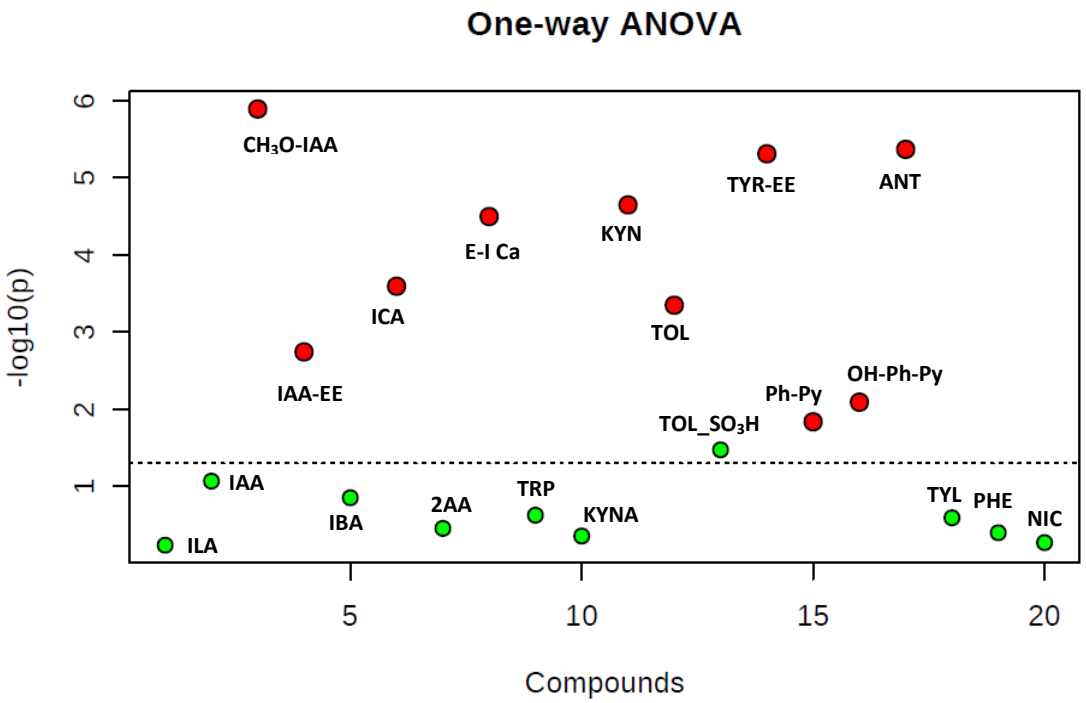
	Compound	g L^{-1}
	Glucose	100
	Fructose	100
Mineral Salts	$CaCl_2$	0.155
	$KH_2 \cdot PO_4$	0.75
	K_2SO_4	0.5
	$MgSO_4 \cdot 7H_2O$	0.25
	NaCl	0.2
	NH_4Cl	0.46
Trace Elements	$COCl_2 \cdot 6H_2O$	0.4
	$CuSO_4 \cdot 5H_2O$	1
	H_3BO_3	1
	KI	1
	$MnSO_4 \cdot H_2O$	4
	$(NH_4)6Mo_7O_{24}$	1
	$ZnSO_4 \cdot H_2O$	4
Nitrogen sources		% wt/wt
	ammoniacal nitrogen 18.6% wt/wt	18.6
	NH_4Cl	20.5
	L-proline	16.9
	L-glutamine	1.25
	L-arginine	6
	L-tryptophan	4.9
	L-alanine	4
	L-glutamic acid	2.6
	L-serine	2.6
	L-threonine	1.6
	L-leucine	1.5
	L-aspartic acid	1.5
	L-valine	1.3
	L-phenylalanine	1.1
	L-isoleucine	1.1
	L-histidine	1.1
	L-methionine	0.6
	L-tyrosine	0.6
	L-glycine	0.6
	L-lysine	0.6
Aerobics Factors		g 100 mL^{-1}
	Oleic acid	0.5
	Ergosterol	1.5
	Tween 80	0.5
Vitamins		mg mL^{-1}
	Biotin	0.003
	Calcium pantothenate	1.5
	Chlorohydrate pyridoxine	0.25
	Chlorohydrate thiamine	0.25
	Myoinositol	20
	Nicotinic acid	2

Supporting Information Table S3. Calibration curve information. The parameters were calculated by the TargetLynx tool of the MassLynx software, and the injection volume was 2 µL.

#	Metabolite	Degree of linearity	LOD (µg L^{-1})	LOQ (µg L^{-1})	*a* (slope)	*b*	R^2
1	nicotinamide (NIC)	5	1.39	4.2	26513.9	-42.33	0.9968
2	5-OH-tryptophan (OH-TRP)	5	1.29	3.90	52940.5	304.35	0.9937
3	5-CH_3O-tryptophan (CH_3O-TRP)	4	3.47	10.5	122841	483.08	0.9836
4	kynurenine (KYN)	5	5.41	16.40	42790.8	526.02	0.9973
5	phenylalanine (PHE)	4	14.82	44.9	249486	17879.20	0.9286
6	3-nitrotyrosine (IS)	5	1.29	3.90	177335	187.22	0.9481
7	4-OH-phenyl pyruvic acid (OH-Ph-Py)	2	391.35	1185.9	3300.82	2873.89	0.9436
8	3-OH-anthranilic acid (OH-ANT)	5	1.06	3.2	72356	466.56	0.8761
9	tryptophan (TRP)	4	3.70	11.2	98314.8	4081.96	0.9877
10	tyrosine ethyl ester (TYR-EE)	3.5	39.4	13.00	369979	2051.90	0.8211
11	N-acetyl serotonin (N-SER)	6	3.00	9.1	44447.7	978.03	0.9233
12	tyrosol (TYL)	4	11.88	36	2436.93	1894.53	0.9762
13	tryptophol sulphonate (TOL-SO_3H)	4	1.39	4.2	4381.18	121.92	0.9531
14	kynurenic acid (KYNA)	3	3.40	10.30	23423.8	2061.11	0.9259
15	4-OH-phenyl acetic acid (OH-Ph-AA)	4	416.57	1262.33	3300.82	2873.89	0.9714
16	tryptophan methyl ester (TRP-ME)	4	72.9	24.06	252041	243204	0.9464
17	phenyl pyruvic acid (Ph-Py)	4	14.26	0.24	302509	-111.49	0.9730
18	indole pyruvic acid (IPy)	3	0.77	0.77	10091	9560.45	0.9977
19	anthranilic acid (ANT)	4	3.10	9.4	110637	729.00	0.9900
20	tryptophan ethyl ester (TRP-EE)	4	82.9	27.36	174531	574258	0.9666
21	phenyl lactic acid (Ph-LA)	3	68.81	208.5	1784.52	155.74	0.9986
22	3-indole lactic acid (ILA)	4	0.14	0.45	105262	46607514	0.9969
23	N-acetyl-L-tyrosine ethyl ester (N-TYR-EE)	5	0.56	1.7	384108	155.93	0.9970
24	indole carboxaldehyde (ICA)	5	0.03	0.09	666814	-11821468	0.9996
25	melatonin (MEL)	5	0.21	0.65	189985	-122.89	0.9993
26	5-CH_3O-indole acetic acid (CH_3O-IAA)	5	0.05	0.16	801.95	37458.61	0.9991

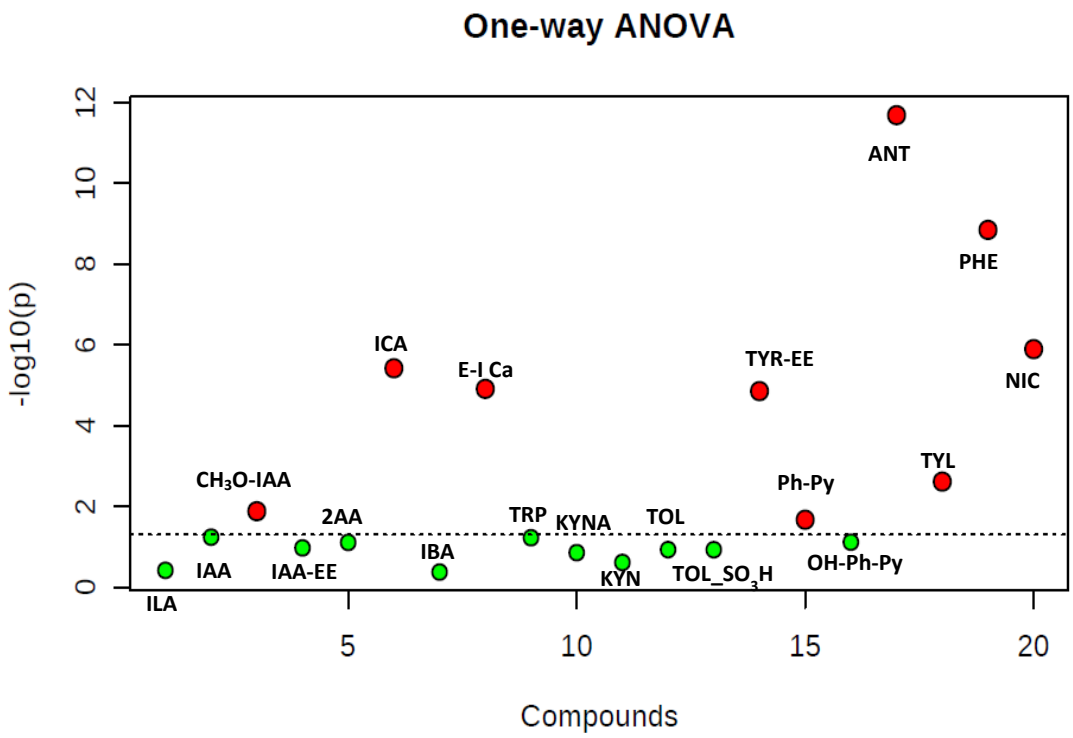
Supporting Information Table S3. Continued

#	Metabolite	Degree of linearity	LOD (µg L^{-1})	LOQ (µg L^{-1})	*a* (slope)	*b*	R^2
27	tryptophol (TOL)	5	23.43	71.00	39496.2	2187.56	0.9758
28	3-indole acetic acid (IAA)	5	0.17	0.52	31.7	18572.02	0.9932
29	phenyl acetic acid (Ph-AA)	3	55.08	166.9	4120.67	1687.99	0.9133
30	2-aminoacetophenone (2AA)	4	0.04	0.15	734168	6005504	0.9995
31	indole (IND)	3	0.06	0.19	10736	-5728107	0.9989
32	3-indole propionic acid (IPA)	5	0.06	0.19	282.95	38674.00	0.9988
33	N-acetyl tryptophan ethyl ester (N-TRP-EE)	5	1.5	0.50	146982	3.48	0.9994
34	3-indole butyric acid (IBA)	4	0.02	0.08	133.08	-1775.78	0.9997
35	methyl-indole acetic acid (M-IAA)	5	0.9	2.8	901707	784.32	0.9973
36	3-CH_3-indole (CH_3-IND)	4	2.18	6.60	89361.3	589.29	0.9942
37	3-indole acetic acid ethyl ester (IAA-EE)	5	0.02	0.06	907.12	495.01	0.9998
38	3-ethyl-indole carboxylic acid (E-ICa)	5	0.06	0.18	495659	-21751351	0.9988



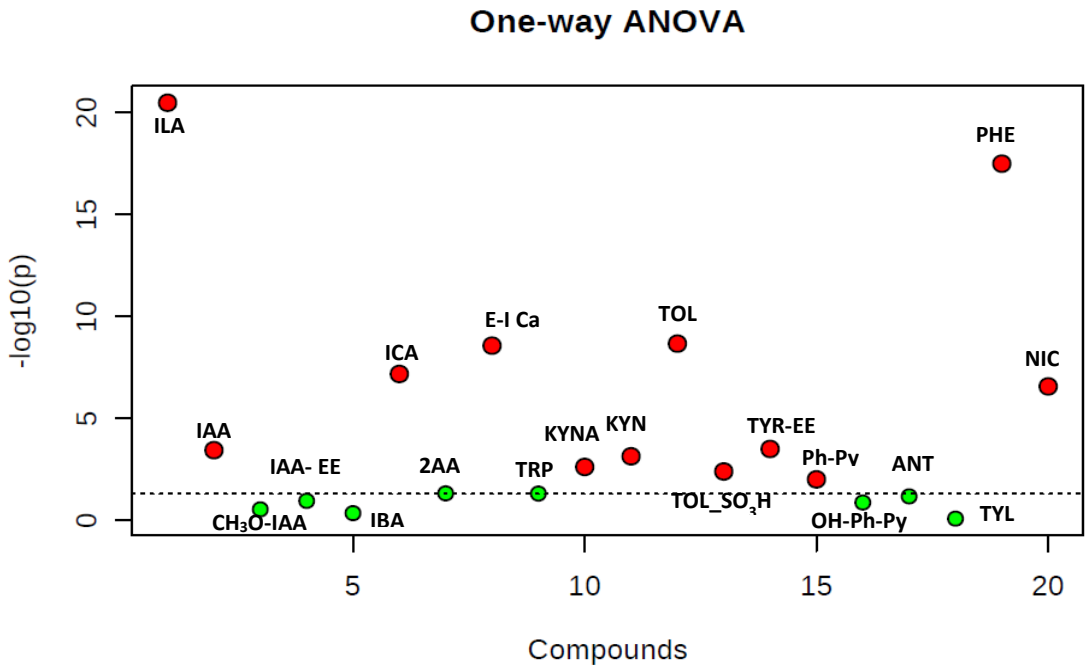
Name	f.value	p-value	-log10(p)	FDR	Tukey's HSD
CH_3O-IAA	25.975	1.0403e-07	6.9828	2.0806e-06	3-1; 3-2
E-I Ca	17.104	6.0035e-06	5.2216	6.0035e-05	3-1; 3-2
ANT	10.443	0.00026508	3.5766	0.0015412	2-1; 3-1
KYN	10.206	0.00030824	3.5111	0.0015412	2-1; 3-1
TYR-EE	9.3655	0.00053117	3.2748	0.0018477	2-1; 3-1
TOL	9.3008	0.0005543	3.2563	0.0018477	2-1; 3-2
ICA	6.9755	0.0027521	2.5603	0.0078631	3-1; 3-2
IAA-EE	6.4943	0.003906	2.4083	0.009765	3-1; 3-2
OH-Ph-Py	4.3867	0.019728	1.7049	0.04384	3-1; 3-2

Supporting Information Figure S1. ANOVA analysis of QA intracellular samples. The points highlighted in red are the compounds with *p*-value≤ 0.05.



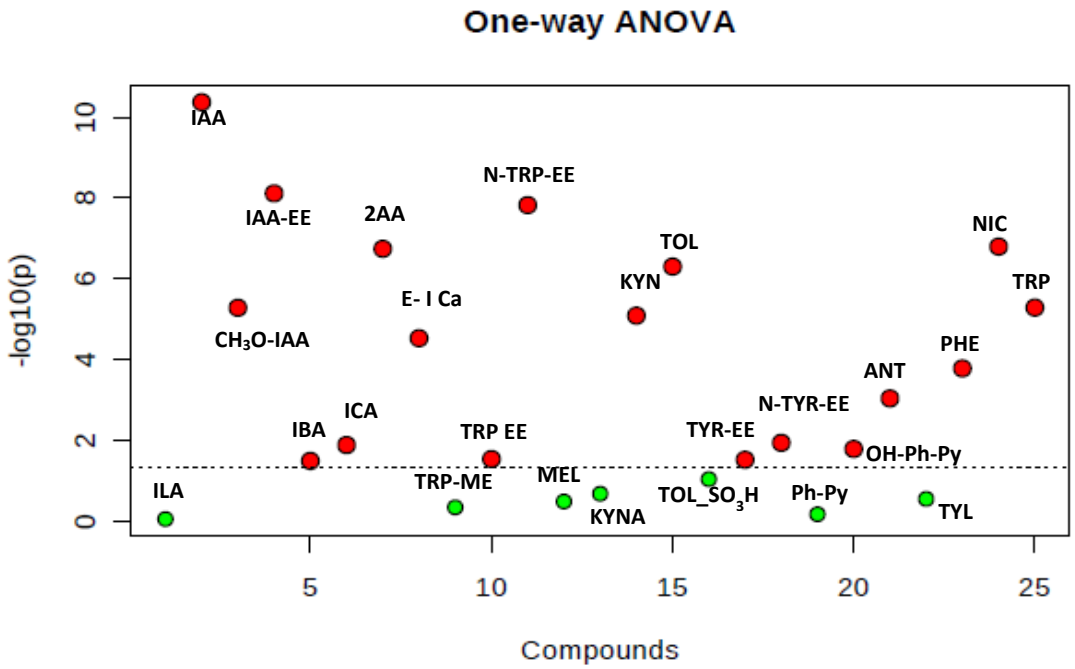
Name	f.value	p.value	-log10(p)	FDR	Tukey's HSD
ANT	63.826	2.1093e-12	11.676	4.2186e-11	2-1; 3-2
PHE	38.478	1.4551e-09	8.8371	1.4551e-08	2-1; 3-1; 3-2
NIC	20.474	1.2949e-06	5.8878	8.6326e-06	3-1; 3-2
ICA	18.18	3.853e-06	5.4142	1.9265e-05	2-1; 3-1; 3-2
E-I Ca	15.867	1.2449e-05	4.9049	4.7112e-05	3-1; 3-2
TYR- EE	15.626	1.4134e-05	4.8497	4.7112e-05	2-1; 3-1
TYL	7.1726	0.0024514	2.6106	0.0070041	2-1; 3-1
CH_3O.IAA	4.9081	0.013215	1.8789	0.033037	3-1; 3-2
Ph-Py	4.2982	0.021419	1.6692	0.047599	3-1

Supporting Information Figure S2. ANOVA analysis of RF intracellular samples. The points highlighted in red are the compounds with p-value$\leq$ 0.05.



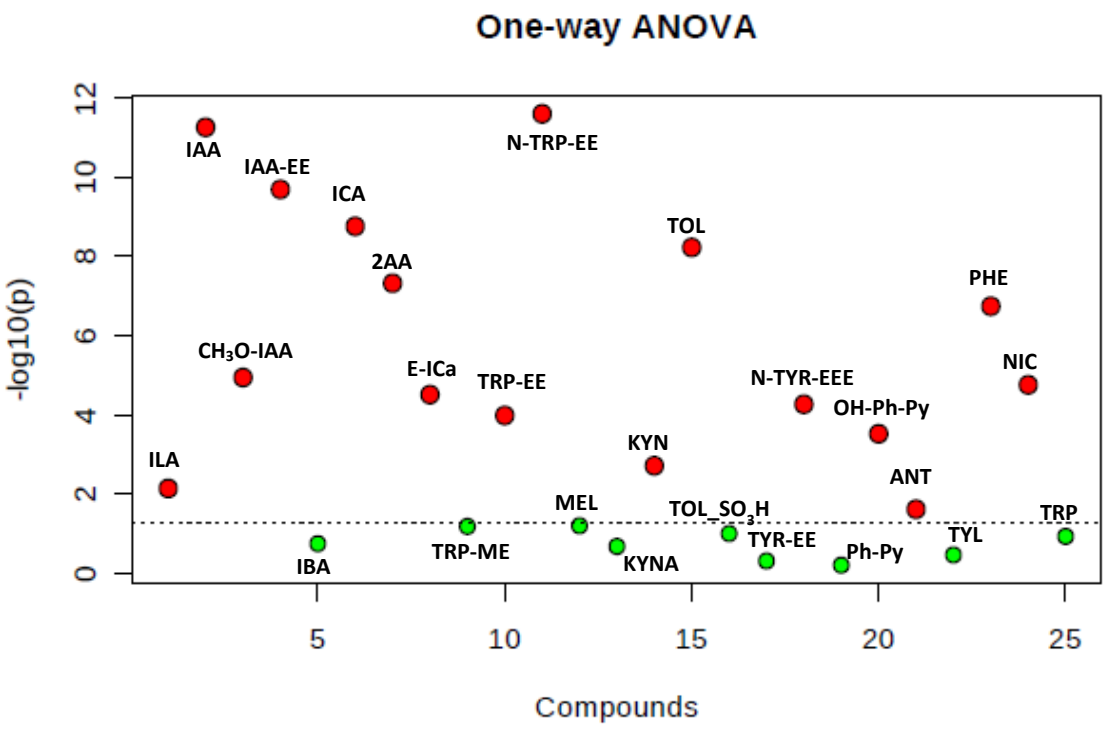
Name	f.value	p.value	-log10(p)	FDR	Tukey's HSD
ILA	217.7	3.4425e-21	20.463	6.8849e-20	2-1; 3-1
PHE	144.41	3.3249e-18	17.478	3.3249e-17	2-1; 3-1
TOL	35.831	2.2098e-09	8.6557	1.3832e-08	2-1; 3-1; 3-2
E-I Ca	35.175	2.7664e-09	8.5581	1.3832e-08	3-1; 3-2
ICA	26.658	6.7632e-08	7.1698	2.7053e-07	2-1; 3-1
NIC	23.347	2.7657e-07	6.5582	9.2191e-07	2-1; 3-2
TYR-EE	10.078	0.00032073	3.4939	0.00091637	2-1; 3-1
IAA	9.8625	0.0003689	3.4331	0.00092224	3-1; 3-2
KYN	8.8237	0.00073568	3.1333	0.0016348	3-2
KYNA	7.1091	0.00244	2.6126	0.0048801	2-1; 3-2
TOL SO_3H	6.4112	0.004068	2.3906	0.0073963	2-1; 3-2
Ph-Py	5.241	0.0099072	2.004	0.016512	2-1; 3-1

Supporting Information Figure S3. ANOVA analysis of *Torulaspora delbrueckii* (Td) intracellular samples. The points highlighted in red are the compounds with p-value≤ 0.05.



Name	f.value	p.value	-log10(p)	FDR	Tukey's HSD
IAA	132.53	4.2757e-11	10.369	1.0689e-09	2-1; 3-1; 3-2
IAA-EE	67.975	7.765e-09	8.1099	9.7062e-08	2-1; 3-1
N-TRP-EE	62.186	1.5161e-08	7.8193	1.2634e-07	2-1; 3-1
NIC	45.007	1.6165e-07	6.7914	9.1852e-07	2-1; 3-1; 3-2
2AA	44.208	1.837e-07	6.7359	9.1852e-07	3-1; 3-2
TOL	38.286	5.0593e-07	6.2959	2.108e-06	3-1; 3-2
TRP	27.028	5.2501e-06	5.2798	1.6564e-05	2-1; 3-2
CH_3O-IAA	26.989	5.3004e-06	5.2757	1.6564e-05	2-1; 3-1
KYN	25.162	8.3059e-06	5.0806	2.3072e-05	3-1; 3-2
E-I Ca	20.419	3.0195e-05	4.5201	7.5488e-05	3-1; 3-2
PHE	15.118	0.00016883	3.7725	0.00038371	2-1; 3-1
ANT	10.809	0.0009357	3.0289	0.0019494	2-1; 3-2
N-TYR-EE	5.834	0.011775	1.9291	0.022643	3-2
ICA	5.6206	0.013375	1.8737	0.023884	3-1
OH-Ph-Py	5.2553	0.016713	1.7769	0.027855	3-1; 3-2
TRP-EE	4.356	0.029693	1.5273	0.045521	2-1
TYR-EE	4.2932	0.030955	1.5093	0.045521	2-1
IBA	4.1915	0.033128	1.4798	0.046011	3-1

Supporting Information Figure S4. ANOVA analysis of QA extracellular samples. The points highlighted in red are the compounds with p-value≤ 0.05.



Name	f.value	p.value	-log10(p)	FDR	Tukey's HSD
N-TRP-EE	217.41	2.5173e-12	11.599	6.2932e-11	2-1; 3-1; 3-2
IAA	196.34	5.519e-12	11.258	6.8987e-11	2-1; 3-1
IAA-EE	122.17	2.0358e-10	9.6913	1.6965e-09	2-1; 3-1
ICA	91.553	1.7389e-09	8.7597	1.0868e-08	2-1; 3-1
TOL	77.391	5.9348e-09	8.2266	2.9674e-08	3-1; 3-2
2AA	57.833	4.7549e-08	7.3229	1.9812e-07	3-1; 3-2
PHE	47.715	1.807e-07	6.7431	6.4534e-07	2-1; 3-1; 3-2
CH_3O-IAA	25.189	1.1396e-05	4.9433	3.5612e-05	2-1; 3-1
NIC	23.476	1.7414e-05	4.7591	4.8373e-05	2-1; 3-1; 3-2
E-I Ca	21.31	3.0801e-05	4.5114	7.7003e-05	3-1; 3-2
N-TYR-EE	19.331	5.3888e-05	4.2685	0.00012247	3-1; 3-2
TRP-EE	17.195	0,00010333	3.9858	0.00021526	3-1; 3-2
OH-Ph-Py	14,061	0.00029904	3.5243	0.00057508	2-1; 3-1
KYN	9.4862	0.0019194	2.7168	0.0034274	3-1; 3-2
ILA	6.8293	0.0071739	2.1442	0.011957	3-1; 3-2
ANT	4.75	0.024024	1.6194	0.037538	3-1

Supporting Information Figure S5. ANOVA analysis of RF extracellular samples. The points highlighted in red are the compounds with p-value≤ 0.05.

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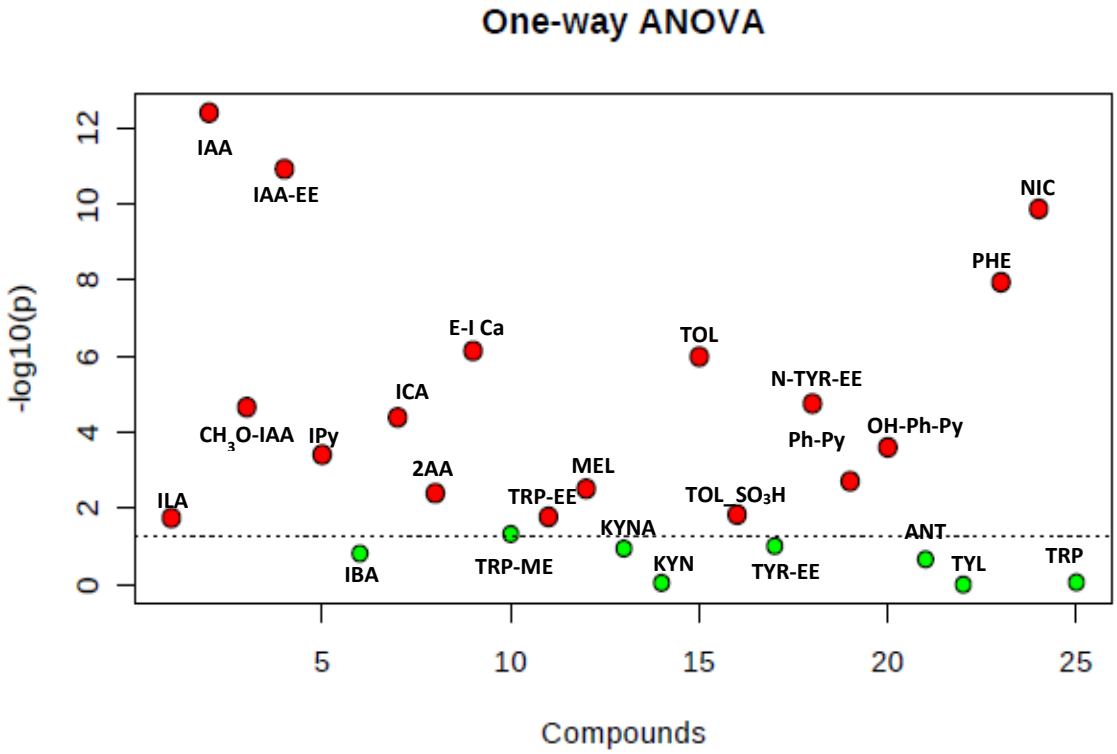
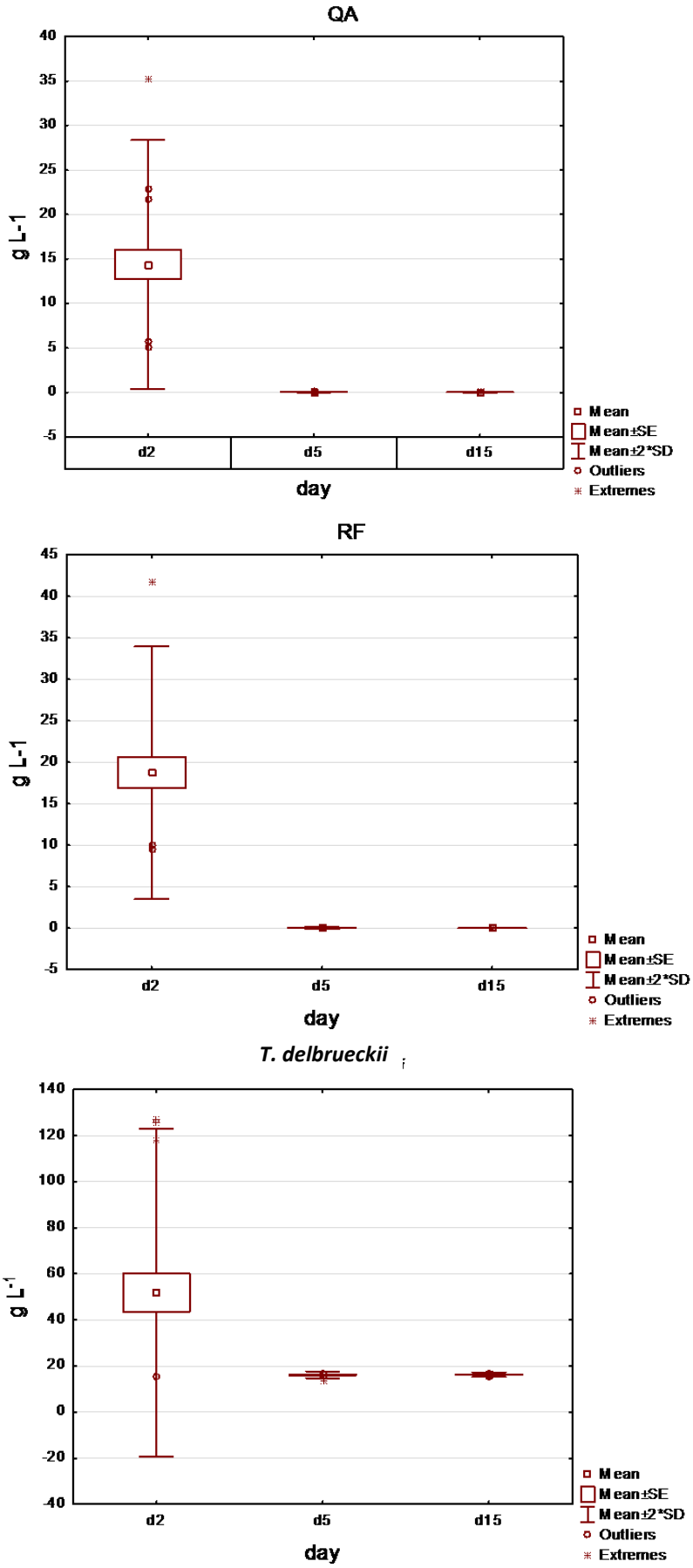
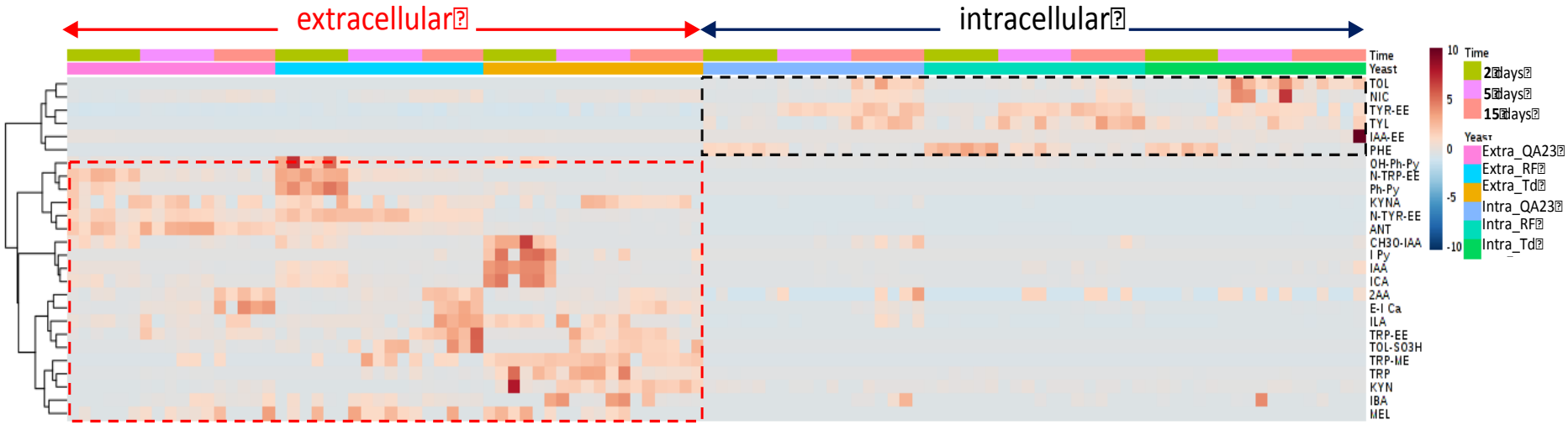


Name	f.value	p.value	-log10(p)	FDR	Tukey's HSD
IAA	276.19	3.9428e-13	12.404	9.8571e-12	2-1; 3-1
IAA-EE	177.6	1.1913e-11	10.924	1.4892e-10	2-1; 3-1
NIC	129.25	1.332e-10	9.8755	1.11e-09	2-1; 3-1; 3-2
PHE	70.818	1.1264e-08	7.9483	7.0401e-08	2-1; 3-1
E-I Ca	38.892	7.1768e-07	6.1441	3.5884e-06	2-1; 3-1; 3-2
TOL	36.875	1.0202e-06	5.9913	4.2507e-06	3-1; 3-2
N-TYR-EE	23.486	1.7371e-05	4.7602	6.2039e-05	2-1; 3-1; 3-2
CH_3O-IAA	22.628	2.1665e-05	4.6642	6.7702e-05	2-1; 3-1
ICA	20.347	4.0243e-05	4.3953	0.00011179	2-1; 3-1
OH-Ph-Py	14,604	0.00024618	3.6087	0.00061546	2-1; 3-1
IPy	13.397	0.00038181	3.4182	0.00086774	2-1; 3-1
Ph-Py	9.4916	0.0019146	2.7179	0.0039888	2-1; 3-1
MEL	8.5267	0.0030146	2.5208	0.0057974	3-1
2AA	7.9826	0.0039404	2.4045	0.0070365	3-1; 3-2
TOL_SO_3H	5.5924	0.0144	1.8416	0.024	2-1; 3-1
TRP-EE	5.3645	0.016486	1.7829	0.025759	2-1
ILA	5.2593	0.017561	1.7554	0.025825	3-2

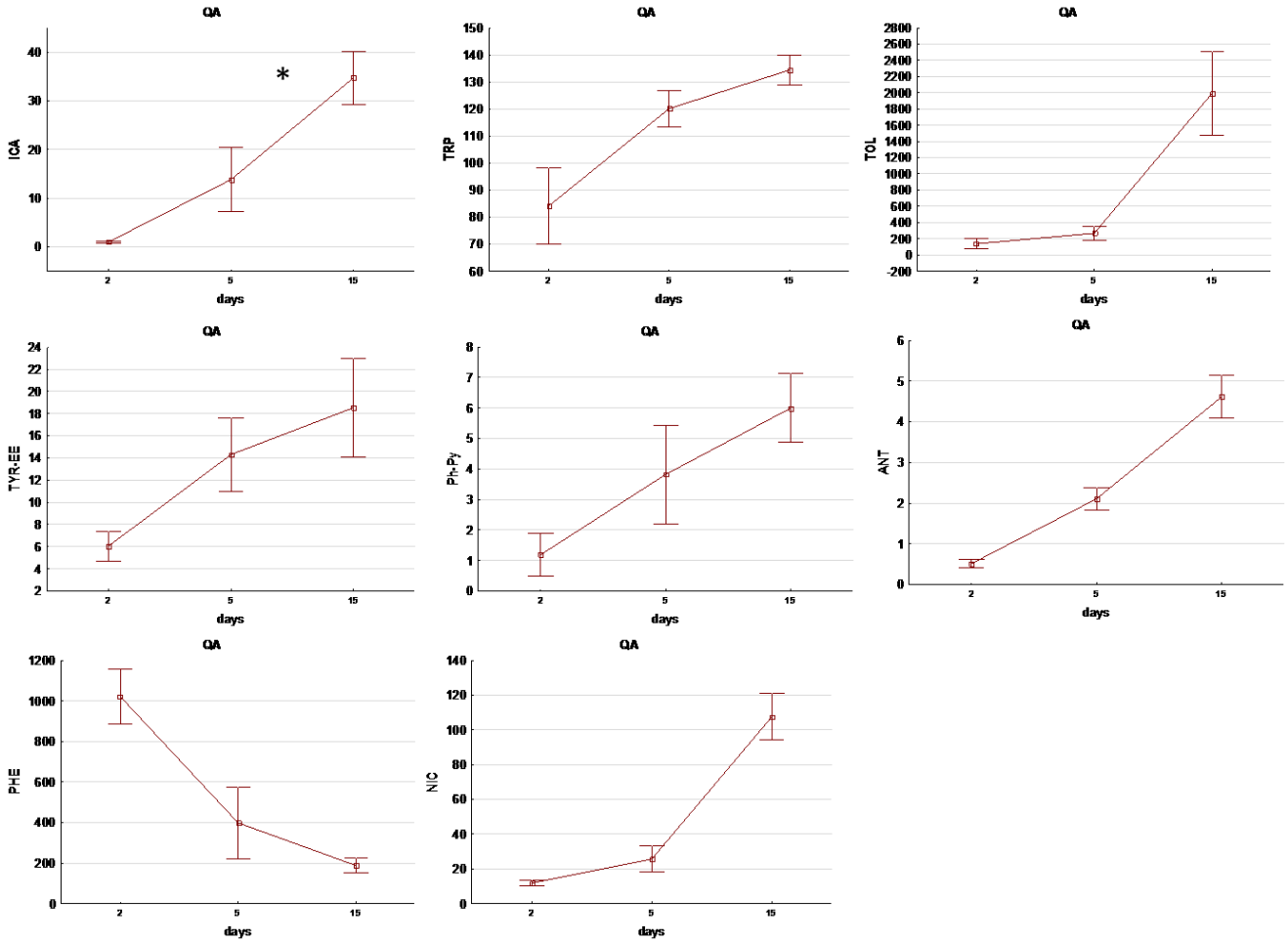
Supporting Information Figure S6. ANOVA analysis of *Torulaspora delbrueckii* (Td) extracellular samples. The points highlighted in red are the compounds with p-value≤ 0.05.



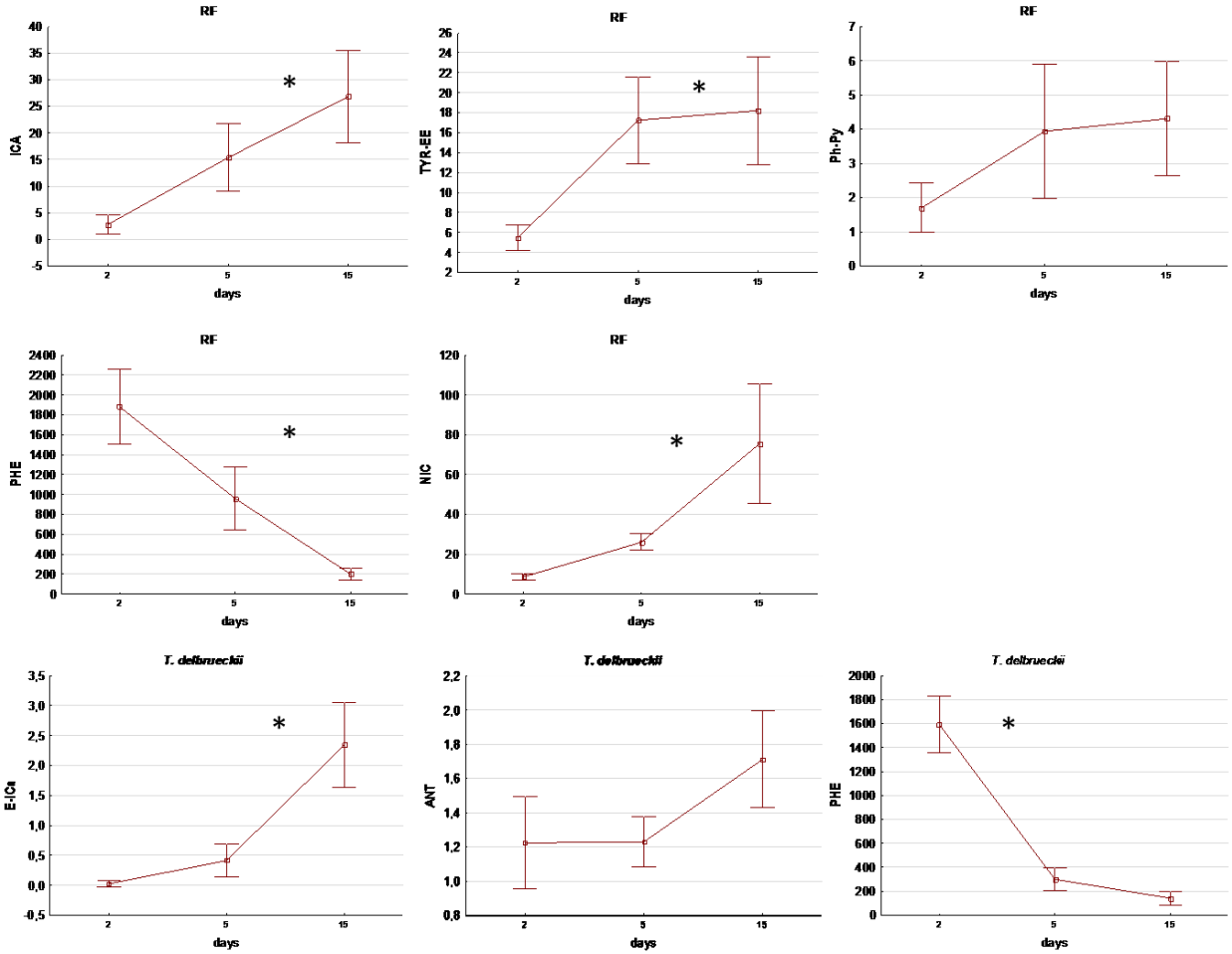
Supporting Information Figure S7. Graphic representation of variability of reducing sugar consumption through sampling time of the six folds of the three strain of yeast studied



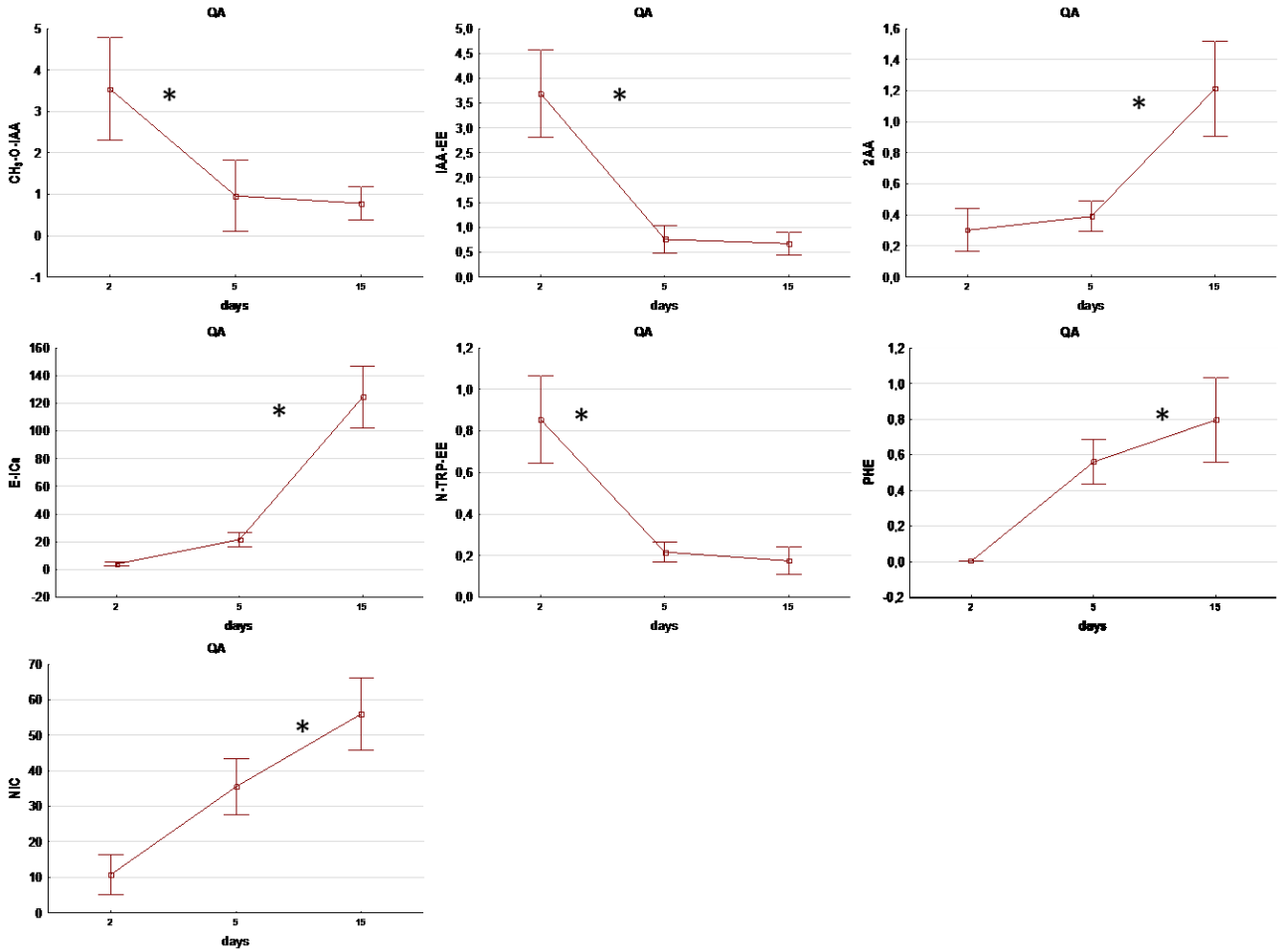
Supporting Information Figure S8. Clustered heatmap of the measured metabolites including both extracellular and intracellular samples of the three yeast strains (the Saccharomyces cerevisiae strains QA23 and RF, and the Torulaspora delbrueckii Td) at three time points of the fermentation process (2, 5 and 15 days). This figure shows it was quantified many more compounds in extracellular samples than in intracellular samples.



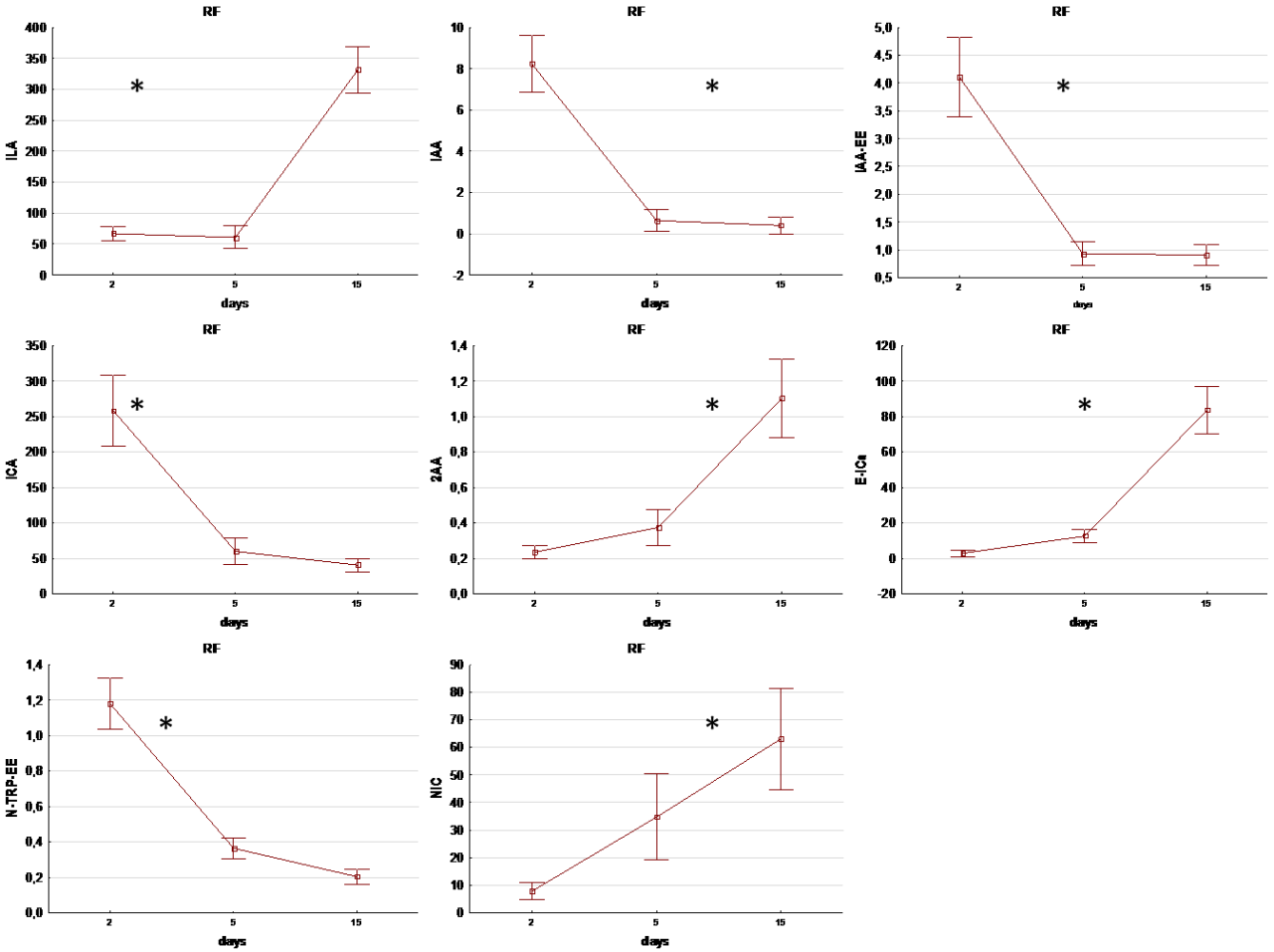
Supporting Information Figure S9. Kinetics of selected compounds of QA intracellular samples. *significant differences ($p<0.05$). The concentration is expressed in µg L^{-1}.



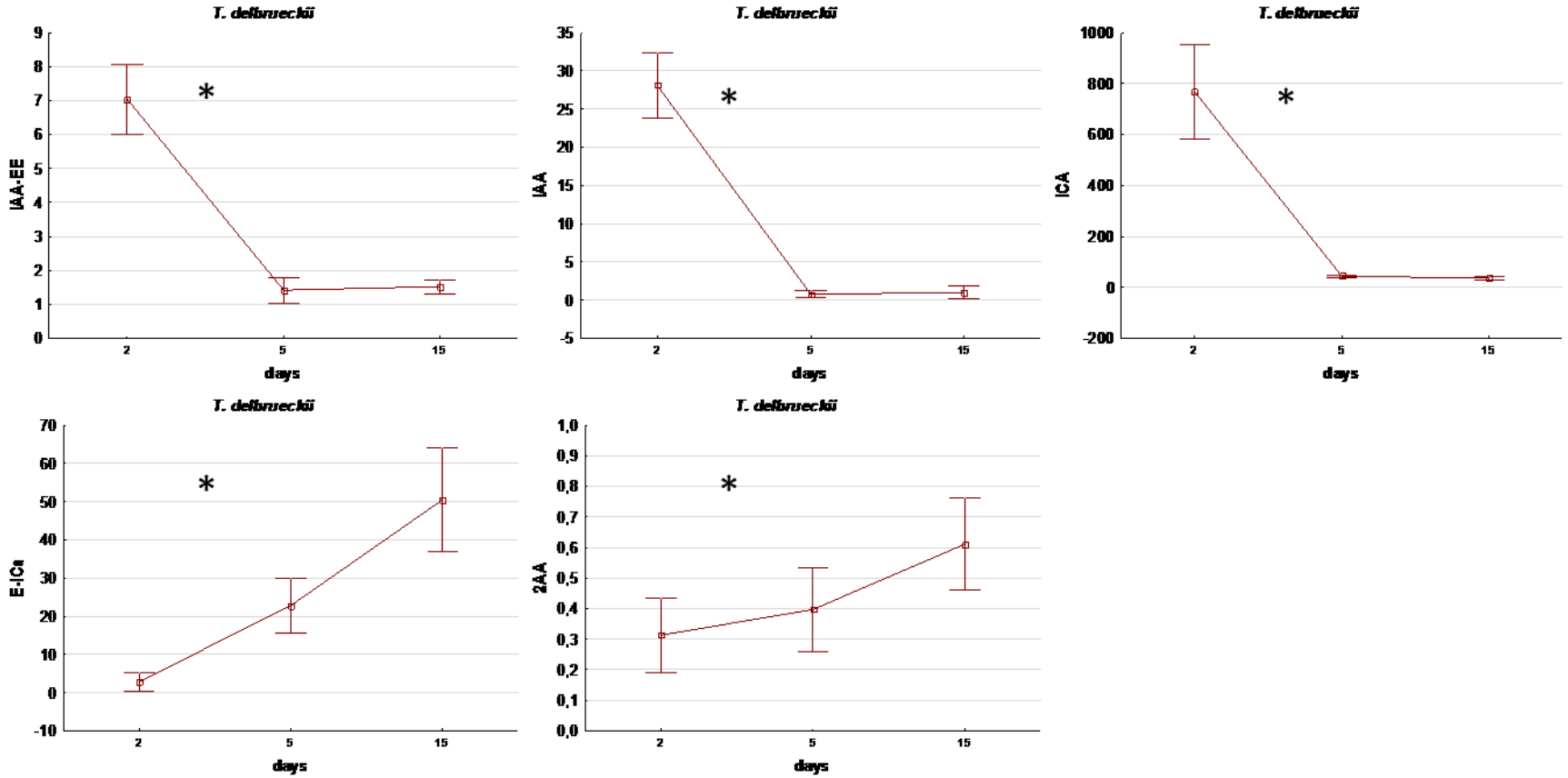
Supporting Information Figure S10. Kinetic of selected compounds of RF and *Torulaspora. delbrueckii* (Td.) intracellular samples. *significant differences ($p<0.05$). The concentration is expressed in μg L^{-1}.



Supporting Information Figure S11. Kinetics of selected compounds of QA extracellular samples. *significant differences ($p<0.05$). The concentration is expressed in μg L^{-1}.



Supporting Information Figure S12. Kinetics of selected compounds of RF extracellular samples. *significant differences ($p<0.05$). The concentration is expressed in µg L^{-1}.



Supporting Information Figure S13. Kinetics of selected compounds of the *Torulaspora delbrueckii* (Td) extracellular samples. *significant differences ($p<0.05$). The concentration is expressed in μg L^{-1}.