Article

Membrane Lipid Composition and Amino Acid Excretion Patterns of *Methanothermococcus Okinawensis* grown in the Presence of Inhibitors Detected in the Enceladian Plume

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Table S1: Elution gradient applied for the separation of primary dissolved free amino acids by high performance liquid chromatography (HPLC). Eluent A (polar phase): 40 mM NaH_2PO_4 buffer (pH 7.8 adjusted with NaOH pellets in Milli-Q water; Sigma-Aldrich); B (non-polar phase): 1000/100/2—Methanol (HPLC grade, Sigma-Aldrich)/Milli-Q water/Trifluoroacetic acid (HPLC grade, Roth); C: Tetrahydrofuran (HPLC grade; Sigma-Aldrich), total time: 90 min.

Time (min)	A %	B %	C %
0	90	10	0
2	90	7.5	2.5
40	68	30	2.0
42	64	35	1.0
75	35	65	0
77	0	100	0
80	90	10	0
90	90	10	0

Table S2: ANOVA analysis of DoE settings. Significant model for OD_{max}.

OD_{max}
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.88	5	0.7752	320.51	**< 0.0001**	significant
A-NH_4Cl	0.7727	1	0.7727	319.46	**< 0.0001**	
B- H_2CO (37%)	2.41	1	2.41	996.27	**< 0.0001**	
AB	0.5444	1	0.5444	225.09	**< 0.0001**	
A^2	0.0335	1	0.0335	13.87	**0.0006**	
B^2	0.0966	1	0.0966	39.93	**< 0.0001**	
Residual	0.0919	38	0.0024			
Lack of Fit	0.0617	9	0.0069	6.57	< 0.0001	significant
Pure Error	0.0302	29	0.0010			
Cor Total	3.97	43				

Fit Statistics

Std. Dev.	0.0492	**R^2**	0.9768
Mean	0.9833	**Adjusted R^2**	0.9738
C.V. %	5.00	**Predicted R^2**	0.9671
		Adeq Precision	48.5543

Final Equation in Terms of Actual Factors

$Sqrt(OD_{max})$ =

+0.890366

+0.075335 NH_4Cl

+0.004703 H_2CO (37%)

-0.000685 NH_4Cl * H_2CO (37%)

-0.002394 NH_4Cl^2

-0.000016 H_2CO (37%)^2

Table S3: Mean values of the OD_{max}, the turnover rate max, and of each lipid in percent of total lipids of all samples of one experiment. For the OD_{max} and the turnover rate max triplicates (n = 3) were used except for "F" (n = 2). Further, for the lipid analysis, one datapoint of each setting "E", "J", and "N" had to be excluded (n=2). Values of extreme values setting are mean values of four samples (n = 4).

DoE

	K	E	B	F	A	I	G	C	M	N	O	J	D	L	H
OD_{max}	1.71	1.62	1.53	1.52	1.51	1.27	1.17	1.16	1.13	1.11	1.10	0.59	0.21	0.20	0.20
Turnover rate max [h^{-1}]	0.096	0.096	0.096	0.095	0.096	0.098	0.098	0.098	0.096	0.097	0.093	0.086	0.031	0.028	0.029
Archaeol	64.5%	68.3%	63.8%	64.4%	63.0%	63.4%	75.4%	72.6%	71.0%	68.7%	72.9%	71.0%	69.1%	74.0%	68.6%
Macr. Ar.	29.7%	25.7%	30.2%	28.8%	29.2%	30.3%	19.3%	19.7%	21.4%	25.1%	18.0%	23.7%	23.5%	18.1%	23.3%
GTGT-0	0.2%	0.3%	0.3%	0.3%	0.4%	0.3%	0.5%	0.7%	0.4%	0.5%	0.6%	0.4%	0.4%	0.8%	0.4%
GDGT-0	3.9%	4.2%	4.1%	4.7%	5.1%	3.7%	3.5%	5.2%	4.7%	3.6%	5.6%	2.7%	4.8%	4.6%	4.5%
GMGT-0	1.1%	1.1%	1.1%	1.2%	1.7%	1.6%	0.8%	1.2%	1.8%	1.5%	2.0%	1.6%	1.6%	1.8%	2.2%
GMGT-0′	0.5%	0.5%	0.4%	0.5%	0.7%	0.8%	0.4%	0.6%	0.7%	0.6%	0.8%	0.7%	0.6%	0.6%	0.9%

Extreme values setting

	Min	Me	Am	Fo
OD_{max}	1.10	1.08	0.52	0.22
Turnover rate max [h^{-1}]	0.094	0.088	0.081	0.051
Archaeol	33.4%	33.5%	41.6%	73.5%
Macr. Ar.	42.3%	44.9%	43.0%	15.5%
GTGT-0	0.3%	0.3%	0.4%	1.1%
GDGT-0	12.6%	9.7%	7.8%	6.9%
GMGT-0	7.6%	7.7%	4.8%	2.1%
GMGT-0′	3.8%	4.0%	2.5%	0.8%

Table S4: ANOVA analysis of DoE settings. Significant model for GTGT-0.

GTGT-0
Transform: Inverse Sqrt
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1018	1	0.1018	4.52	**0.0392**	significant
C-CH_3OH	0.1018	1	0.1018	4.52	**0.0392**	
Residual	0.9912	44	0.0225			
Lack of Fit	0.3624	13	0.0279	1.37	0.2269	not significant
Pure Error	0.6289	31	0.0203			
Cor Total	1.09	45				

Fit Statistics

Std. Dev.	0.1501	**R^2**	0.0931
Mean	0.6682	**Adjusted R^2**	0.0725
C.V. %	22.46	**Predicted R^2**	0.0212
		Adeq Precision	5.4241

Final Equation in Terms of Actual Factors

1/Sqrt(GTGT 0a) =
+0.576726
+0.000841 CH_3OH

Table S5: ANOVA analysis of DoE settings. Significant model for GMGT-0′.

GMGT-0′
Transform: Inverse Sqrt
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2969	6	0.0495	3.22	**0.0115**	significant
A-NH_4Cl	0.0013	1	0.0013	0.0824	0.7757	
B-H_2CO (37%)	0.0220	1	0.0220	1.43	0.2389	
C-CH_3OH	0.0524	1	0.0524	3.41	0.0725	
A^2	0.0332	1	0.0332	2.16	0.1498	
B^2	0.1168	1	0.1168	7.59	**0.0089**	
C^2	0.1397	1	0.1397	9.09	**0.0045**	
Residual	0.5997	39	0.0154			
Lack of Fit	0.2195	8	0.0274	2.24	0.0516	not significant
Pure Error	0.3801	31	0.0123			
Cor Total	0.8966	45				

Fit Statistics

Std. Dev.	0.1240	**R^2**	0.3312
Mean	0.5235	**Adjusted R^2**	0.2283
C.V. %	23.69	**Predicted R^2**	0.0443
		Adeq Precision	5.6268

Final Equation in Terms of Actual Factors

1/Sqrt(GMGT 0a') =	
-0.131956	
+0.048749	NH_4Cl
+0.004564	H_2CO (37%)
+0.005168	CH_3OH
-0.002589	NH_4Cl^2
-0.000019	H_2CO (37%)^2
-0.000021	CH_3OH^2

Table S6: ANOVA analysis of DoE settings. Significant model for GTGT/GMGT (including both GMGT isomers).

GTGT/GMGT
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.3236	3	0.1079	3.22	**0.0321**	significant
A-NH_4Cl	0.0020	1	0.0020	0.0612	0.8058	
B-H_2CO (37%)	0.1654	1	0.1654	4.94	**0.0317**	
AB	0.2809	1	0.2809	8.39	**0.0060**	
Residual	1.41	42	0.0335			
Lack of Fit	0.2739	11	0.0249	0.6817	0.7449	not significant
Pure Error	1.13	31	0.0365			
Cor Total	1.73	45				

Fit Statistics

Std. Dev.	0.1830	**R^2**	0.1871
Mean	-0.7435	**Adjusted R^2**	0.1290
C.V. %	24.61	**Predicted R^2**	0.0224
		Adeq Precision	5.3544

Final Equation in Terms of Actual Factors

Log_{10}(GTGT/GMGT) =

-0.403862

-0.052201 NH_4Cl

-0.003250 H_2CO (37%)

+0.000492 NH_4Cl * H_2CO (37%)

Table S7: ANOVA analysis of DoE settings. Significant model for GMGT-0/0'.

GMGT-0/0'
Transform: Power
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7319	4	0.1830	3.69	**0.0117**	significant
A-NH_4Cl	0.1631	1	0.1631	3.29	0.0770	
B-H_2CO (37%)	0.0035	1	0.0035	0.0697	0.7931	
C-CH_3OH	0.1317	1	0.1317	2.66	0.1108	
BC	0.4229	1	0.4229	8.53	**0.0057**	
Residual	2.03	41	0.0496			
Lack of Fit	0.3604	10	0.0360	0.6681	0.7445	not significant
Pure Error	1.67	31	0.0539			
Cor Total	2.76	45				

Fit Statistics

Std. Dev.	0.2226	**R^2**	0.2647
Mean	2.34	**Adjusted R^2**	0.1930
C.V. %	9.50	**Predicted R^2**	0.0608
		Adeq Precision	7.0124

Final Equation in Terms of Actual Factors

(GMGT 0a/0a')^1 =

+1.84499

+0.017060 NH_4Cl

+0.004308 H_2CO (37%)

+0.003194 CH_3OH

Table S8: ANOVA analysis of DoE settings. Significant model for glutamic acid (Glu).

Glu

Transform: Inverse Sqrt

Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0284	4	0.0071	3.64	**0.0130**	significant
B- H_2CO (37%)	0.0022	1	0.0022	1.14	0.2914	
C-CH_3OH	0.0035	1	0.0035	1.79	0.1891	
BC	0.0075	1	0.0075	3.83	0.0577	
C^2	0.0152	1	0.0152	7.78	**0.0081**	
Residual	0.0761	39	0.0020			
Lack of Fit	0.0174	10	0.0017	0.8597	0.5788	not significant
Pure Error	0.0587	29	0.0020			
Cor Total	0.1044	43				

Fit Statistics

Std. Dev.	0.0442	**R^2**	0.2716
Mean	0.1621	**Adjusted R^2**	0.1969
C.V. %	27.24	**Predicted R^2**	0.0794
		Adeq Precision	5.9661

Final Equation in Terms of Actual Factors

1/Sqrt(Glu) =

+0.201845

-0.000676 H_2CO (37%)

+0.000571 CH_3OH

+5.01076E-06 H_2CO (37%) * CH_3OH

-5.80794E-06 CH_3OH^2

Table S9: ANOVA analysis of DoE settings. Significant model for asparagine (Asn).

Asn

Transform: Square Root

Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.55	4	2.39	3.13	**0.0253**	significant
B- H_2CO (37%)	0.1416	1	0.1416	0.1854	0.6691	
C-CH_3OH	2.11	1	2.11	2.77	0.1041	
BC	3.01	1	3.01	3.94	0.0543	
C^2	4.41	1	4.41	5.78	**0.0211**	
Residual	29.79	39	0.7638			
Lack of Fit	6.54	10	0.6542	0.8162	0.6159	not significant
Pure Error	23.25	29	0.8016			
Cor Total	39.34	43				

Fit Statistics

Std. Dev.	0.8739	**R^2**	0.2429
Mean	1.68	**Adjusted R^2**	0.1652
C.V. %	51.92	**Predicted R^2**	0.0486
		Adeq Precision	5.7417

Final Equation in Terms of Actual Factors

Sqrt(Asn) =

+0.821300

+0.012061 H_2CO (37%)

-0.006891 CH_3OH

-0.000101 H_2CO (37%)* CH_3OH

+0.000099 CH_3OH^2

Table S10: ANOVA analysis of DoE settings. Significant model for serine (Ser).
Ser
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.18	2	0.5878	4.30	**0.0201**	significant
C-CH_3OH	0.2472	1	0.2472	1.81	0.1860	
C^2	0.9306	1	0.9306	6.81	**0.0126**	
Residual	5.60	41	0.1366			
Lack of Fit	1.80	12	0.1496	1.14	0.3685	not significant
Pure Error	3.81	29	0.1313			
Cor Total	6.78	43				

Fit Statistics

Std. Dev.	0.3696	**R^2**	0.1734
Mean	0.3264	**Adjusted R^2**	0.1331
C.V. %	113.24	**Predicted R^2**	0.0627
		Adeq Precision	6.1706

Final Equation in Terms of Actual Factors

$Log_{10}(Ser)$ =
+0.585712
-0.008698 CH_3OH
+0.000045 CH_3OH^2

Table S11: ANOVA analysis of DoE settings. Significant model for glycine (Gly).

Gly

Transform: Base 10 Log

Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.24	4	0.3101	2.94	**0.0325**	significant
B- H_2CO (37%)$_2$	0.0286	1	0.0286	0.2706	0.6059	
C-CH_3OH	0.2754	1	0.2754	2.61	0.1144	
BC	0.3612	1	0.3612	3.42	0.0720	
C^2	0.5880	1	0.5880	5.57	**0.0234**	
Residual	4.12	39	0.1056			
Lack of Fit	0.9094	10	0.0909	0.8219	0.6109	not significant
Pure Error	3.21	29	0.1106			
Cor Total	5.36	43				

Fit Statistics

Std. Dev.	0.3250	**R^2**	0.2315
Mean	1.56	**Adjusted R^2**	0.1527
C.V. %	20.78	**Predicted R^2**	0.0245
		Adeq Precision	5.5980

Final Equation in Terms of Actual Factors

$Log_{10}(Gly)$ =

+1.26420

+0.004282 H_2CO (37%)

-0.002735 CH_3OH

-0.000035 H_2CO (37%)* CH_3OH

+0.000036 CH_3OH^2

Table S12: ANOVA analysis of DoE settings. Significant model for alanine (Ala).
Ala
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.8137	4	0.2034	2.77	**0.0408**	significant
B- H_2CO (37%)	0.0095	1	0.0095	0.1295	0.7209	
C-CH_3OH	0.1778	1	0.1778	2.42	0.1281	
BC	0.2269	1	0.2269	3.08	0.0869	
C^2	0.4099	1	0.4099	5.57	**0.0233**	
Residual	2.87	39	0.0736			
Lack of Fit	0.7646	10	0.0765	1.05	0.4266	not significant
Pure Error	2.10	29	0.0726			
Cor Total	3.68	43				

Fit Statistics

Std. Dev.	0.2712	**R^2**	0.2210
Mean	1.23	**Adjusted R^2**	0.1411
C.V. %	22.08	**Predicted R^2**	0.0077
		Adeq Precision	5.3901

Final Equation in Terms of Actual Factors

$Log_{10}(Ala)$ =

+1.01307

+0.003298 H_2CO (37%)

-0.002490 CH_3OH

-0.000028 H_2CO (37%)* CH_3OH

+0.000030 CH_3OH^2

Table S13: ANOVA analysis of DoE settings. Significant model for tyrosine (Tyr).
Tyr
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	14.35	4	3.59	3.55	**0.0146**	significant
B- H_2CO (37%)	0.2950	1	0.2950	0.2917	0.5922	
C-CH_3OH	1.41	1	1.41	1.40	0.2444	
BC	3.77	1	3.77	3.73	0.0609	
C^2	8.96	1	8.96	8.86	**0.0050**	
Residual	39.44	39	1.01			
Lack of Fit	12.51	10	1.25	1.35	0.2534	not significant
Pure Error	26.94	29	0.9288			
Cor Total	53.80	43				

Fit Statistics

Std. Dev.	1.01	**R^2**	0.2668
Mean	1.68	**Adjusted R^2**	0.1916
C.V. %	59.76	**Predicted R^2**	0.0658
		Adeq Precision	5.6803

Final Equation in Terms of Actual Factors

Sqrt(Tyr) =
+1.07569
+0.013822 H_2CO (37%)
-0.015538 CH_3OH
-0.000113 H_2CO (37%)* CH_3OH
+0.000141 CH_3OH^2

Table S14: ANOVA analysis of DoE settings. Significant model for tryptophan (Trp).

Trp
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.40	4	0.3488	3.33	**0.0194**	significant
B- H_2CO (37%)	0.0360	1	0.0360	0.3430	0.5615	
C-CH_3OH	0.2690	1	0.2690	2.57	0.1172	
BC	0.4041	1	0.4041	3.86	0.0568	
C^2	0.6985	1	0.6985	6.66	**0.0137**	
Residual	4.09	39	0.1048			
Lack of Fit	0.8849	10	0.0885	0.8012	0.6287	not significant
Pure Error	3.20	29	0.1104			
Cor Total	5.48	43				

Fit Statistics

Std. Dev.	0.3237	**R^2**	0.2545
Mean	0.0349	**Adjusted R^2**	0.1780
C.V. %	927.81	**Predicted R^2**	0.0588
		Adeq Precision	5.8875

Final Equation in Terms of Actual Factors

$Log_{10}(Trp)$ =

-0.264752

+0.004557 H_2CO (37%)

-0.003246 CH_3OH

-0.000037 H_2CO (37%)* CH_3OH

+0.000039 CH_3OH^2

Table S15: ANOVA analysis of DoE settings. Significant model for phenylalanine (Phe).
Phe
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.7950	2	0.3975	3.72	**0.0327** significant
C-CH_3OH	0.2231	1	0.2231	2.09	0.1560
C^2	0.5737	1	0.5737	5.37	**0.0255**
Residual	4.38	41	0.1068		
Lack of Fit	1.29	12	0.1078	1.01	0.4627 not significant
Pure Error	3.09	29	0.1064		
Cor Total	5.17	43			

Fit Statistics

Std. Dev.	0.3268	**R^2**	0.1536
Mean	0.8346	**Adjusted R^2**	0.1124
C.V. %	39.16	**Predicted R^2**	0.0463
		Adeq Precision	5.7348

Final Equation in Terms of Actual Factors

$$Log_{10}(Phe) = +1.01473 - 0.006613\ CH_3OH + 0.000036\ CH_3OH^2$$

Table S16: ANOVA analysis of DoE settings. Significant model for total amino acids.
Total Amino acids
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.01	4	0.2532	3.18	**0.0236**	significant
B- H_2CO (37%)	0.0344	1	0.0344	0.4325	0.5146	
C-CH_3OH	0.2325	1	0.2325	2.92	0.0954	
BC	0.2675	1	0.2675	3.36	0.0745	
C^2	0.4862	1	0.4862	6.11	**0.0180**	
Residual	3.11	39	0.0796			
Lack of Fit	0.8048	10	0.0805	1.01	0.4553	not significant
Pure Error	2.30	29	0.0793			
Cor Total	4.12	43				

Fit Statistics

Std. Dev.	0.2822	**R^2**	0.2459
Mean	2.24	**Adjusted R^2**	0.1686
C.V. %	12.61	**Predicted R^2**	0.0367
		Adeq Precision	5.8731

Final Equation in Terms of Actual Factors

Log_{10}(Total Amino acids) =

+1.97501

+0.003791 H_2CO (37%)

-0.002661 CH_3OH

-0.000030 H_2CO (37%)* CH_3OH

+0.000033 CH_3OH^2

Table S17: ANOVA analysis of DoE settings. Significant model for glutamine (Gln).

Gln
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.36	2	2.18	4.57	**0.0162**	significant
A-NH_4Cl	2.85	1	2.85	5.96	**0.0190**	
C- CH_3OH	1.62	1	1.62	3.39	0.0727	
Residual	19.57	41	0.4772			
Lack of Fit	4.09	12	0.3411	0.6393	0.7916	not significant
Pure Error	15.47	29	0.5336			
Cor Total	23.92	43				

Fit Statistics

Std. Dev.	0.6908	**R^2**	0.1822
Mean	1.51	**Adjusted R^2**	0.1423
C.V. %	45.66	**Predicted R^2**	0.0593
		Adeq Precision	5.1932

Final Equation in Terms of Actual Factors

Sqrt(Gln) =
+2.42325
-0.071202 NH_4Cl
-0.003356 CH_3OH

Table S18: ANOVA analysis of DoE settings. Significant model for arginine (Arg).

Arg
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.71	1	1.71	5.75	**0.0210**	significant
A-NH_4Cl	1.71	1	1.71	5.75	**0.0210**	
Residual	12.50	42	0.2976			
Lack of Fit	3.18	13	0.2450	0.7628	0.6900	not significant
Pure Error	9.31	29	0.3212			
Cor Total	14.21	43				

Fit Statistics

Std. Dev.	0.5455	**R^2**	0.1205
Mean	0.7682	**Adjusted R^2**	0.0995
C.V. %	71.01	**Predicted R^2**	0.0432
		Adeq Precision	5.9867

Final Equation in Terms of Actual Factors

Sqrt(Arg) =

+0.345122

+0.055201 NH_4Cl

Table S19: ANOVA analysis of DoE settings. Significant model for valine (Val).

Val
Transform: Inverse Sqrt
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0563	1	0.0563	5.68	**0.0217** significant
A-NH_4Cl	0.0563	1	0.0563	5.68	**0.0217**
Residual	0.4159	42	0.0099		
Lack of Fit	0.1203	13	0.0093	0.9076	0.5561 not significant
Pure Error	0.2956	29	0.0102		
Cor Total	0.4722	43			

Fit Statistics

Std. Dev.	0.0995	**R^2**	0.1191
Mean	0.3033	**Adjusted R^2**	0.0982
C.V. %	32.81	**Predicted R^2**	0.0393
		Adeq Precision	5.9494

Final Equation in Terms of Actual Factors

1/Sqrt(Val) =
+0.380002
-0.010007 NH_4Cl

Table S20: ANOVA analysis of DoE settings. Significant model for isoleucine (Ile).

Ile
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.37	5	0.2741	3.13	**0.0183**	significant
A-NH_4Cl	0.4420	1	0.4420	5.05	**0.0305**	
B- H_2CO (37%)	0.0020	1	0.0020	0.0224	0.8819	
C- CH_3OH	0.2655	1	0.2655	3.04	0.0895	
BC	0.3051	1	0.3051	3.49	0.0695	
C^2	0.4161	1	0.4161	4.76	**0.0354**	
Residual	3.32	38	0.0875			
Lack of Fit	0.6451	9	0.0717	0.7760	0.6394	not significant
Pure Error	2.68	29	0.0924			
Cor Total	4.69	43				

Fit Statistics

Std. Dev.	0.2958	**R^2**	0.2919
Mean	1.08	**Adjusted R^2**	0.1988
C.V. %	27.38	**Predicted R^2**	0.0449
		Adeq Precision	5.9240

Final Equation in Terms of Actual Factors

$Log_{10}(Ile)$ =

+0.587403

+0.028086 NH_4Cl

+0.003644 H_2CO (37%)

-0.001805 CH_3OH

-0.000032 H_2CO (37%)* CH_3OH

+0.000030 CH_3OH^2

Table S21: ANOVA analysis of DoE settings. Significant model for leucine (Leu).

Leu
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.35	5	0.2700	3.04	**0.0210**	significant
A-NH4Cl	0.3884	1	0.3884	4.37	**0.0432**	
B- H_2CO (37%)	0.0024	1	0.0024	0.0273	0.8696	
C- CH_3OH	0.2784	1	0.2784	3.13	0.0847	
BC	0.3009	1	0.3009	3.39	0.0735	
C^2	0.4380	1	0.4380	4.93	**0.0324**	
Residual	3.37	38	0.0888			
Lack of Fit	0.6405	9	0.0712	0.7547	0.6573	not significant
Pure Error	2.73	29	0.0943			
Cor Total	4.73	43				

Fit Statistics

Std. Dev.	0.2980	**R^2**	0.2857
Mean	1.02	**Adjusted R^2**	0.1918
C.V. %	29.23	**Predicted R^2**	0.0391
		Adeq Precision	5.9139

Final Equation in Terms of Actual Factors

$Log_{10}(Leu)$ =

+0.544955

+0.026329 NH4Cl

+0.003633 H_2CO (37%)

-0.001971 CH_3OH

-0.000032 H_2CO (37%)* CH_3OH

+0.000031 CH_3OH^2

Table S22: ANOVA analysis of DoE settings. Significant model for threonine (Thr).

Thr
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.73	4	1.68	3.21	**0.0227**	significant
B- H_2CO (37%)	0.7547	1	0.7547	1.44	0.2373	
C- CH_3OH	1.14	1	1.14	2.18	0.1478	
BC	2.25	1	2.25	4.29	**0.0450**	
C^2	2.57	1	2.57	4.90	**0.0328**	
Residual	20.44	39	0.5240			
Lack of Fit	5.39	10	0.5389	1.04	0.4376	not significant
Pure Error	15.05	29	0.5189			
Cor Total	27.17	43				

Fit Statistics

Std. Dev.	0.7239	**R^2**	0.2477
Mean	1.56	**Adjusted R^2**	0.1705
C.V. %	46.39	**Predicted R^2**	0.0220
		Adeq Precision	5.7448

Final Equation in Terms of Actual Factors

Sqrt(Thr) =

+0.620539

+0.011860 H_2CO (37%)

-0.004232 CH_3OH

-0.000087 H_2CO (37%)* CH_3OH

+0.000076 CH_3OH^2

Table S23: ANOVA analysis of DoE settings. Significant model for aspartic acid (Asp).

Asp
Transform: Inverse

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0117	2	0.0058	3.45	**0.0411**	significant
C- CH_3OH	0.0052	1	0.0052	3.06	0.0878	
C^2	0.0065	1	0.0065	3.86	0.0561	
Residual	0.0693	41	0.0017			
Lack of Fit	0.0200	12	0.0017	0.9778	0.4912	not significant
Pure Error	0.0493	29	0.0017			
Cor Total	0.0809	43				

Fit Statistics

Std. Dev.	0.0411	**R^2**	0.1441
Mean	0.1058	**Adjusted R^2**	0.1024
C.V. %	38.87	**Predicted R^2**	0.0280
		Adeq Precision	5.3969

Final Equation in Terms of Actual Factors

1/(Asp) =
+0.092712
+0.000649 CH_3OH
-3.81072E-06 CH_3OH^2

Table S24. Levene-tests (modified robust Brown-Forsythe Levene-type test based on the absolute deviations from the median) of lipid and amino acid production in the extreme values setting (non-significant result if p-value > 0.05, marked in bold). Abbreviations of amino acids: Asp = aspartic acid; Glu = glutamic acid; Asn = asparagine; Ser = serine; Gln = glutamine; His = histidine; Gly = glycine; Thr = threonine; Arg = arginine; Ala = alanine; Tyr = tyrosine; Val = valine; Met = methionine; Trp = tryptophan; Phe = phenylalanine; Ile = isoleucine; Leu = leucine.

Lipids		**Amino Acids**	
ratio	**p-value**	**species**	**p-value**
Archaeol/macrocycle	4.72e-07	**Asp**	**0.07071**
Diethers/tetraethers	**0.486**	**Glu**	0.001651
GDGT/GMGT	**0.2931**	**Asn**	2.315e-05
GTGT/GDGT	**0.1927**	**Ser**	0.0111
GTGT/GMGT	0.03613	**Gln**	0.04085
GMGT-0/0′	0.001651	**His**	n.a.
		Gly	0.007693
		Thr	0.01707
		Arg	0.0004998
		Ala	0.0007432
		Tyr	0.0118
		Val	0.005982
		Met	2.173e-05
		Trp	0.03481
		Phe	0.01328
		Ile	0.0002112
		Leu	0.002336
		total	0.008958

Table S25. ANOVA analysis of lipid and amino acid species that showed no significance at the performed Levene test (see Table S26, significant if Pr(>F) < 0.01). Asp = aspartic acid.

Analysis for Diethers/tetraethers

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
NH_4Cl	1	0.112	0.112	0.0450	0.8354766
H_2CO	1	69.295	69.295	27.8926	**0.0001942**
CH_3OH	1	0.029	0.029	0.0119	0.9150552
Residuals	12	29.812	2.484		

Analysis for GDGT/GMGT

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
NH_4Cl	1	0.4919	0.4919	4.1580	0.06409
H_2CO	1	7.7360	7.7360	65.3901	**3.368e-06**
CH_3OH	1	0.1326	0.1326	1.1209	0.31058
Residuals	12	1.4197	0.1183		

Analysis for GTGT-0/GDGT-0

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
NH_4Cl	1	0.002138	0.002138	3.4467	0.08809
H_2CO	1	0.046884	0.046884	75.5676	**1.59e-06**
CH_3OH	1	0.000003	0.000003	0.0040	0.95034
Residuals	12	0.007445	0.000620		

Analysis for Asp

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
NH_4Cl	1	197.030	197.030	11.0624	**0.006043**
H_2CO	1	5.556	5.556	0.3119	0.586766
CH_3OH	1	22.739	22.739	1.2767	0.280599
Residuals	12	213.730	17.811		

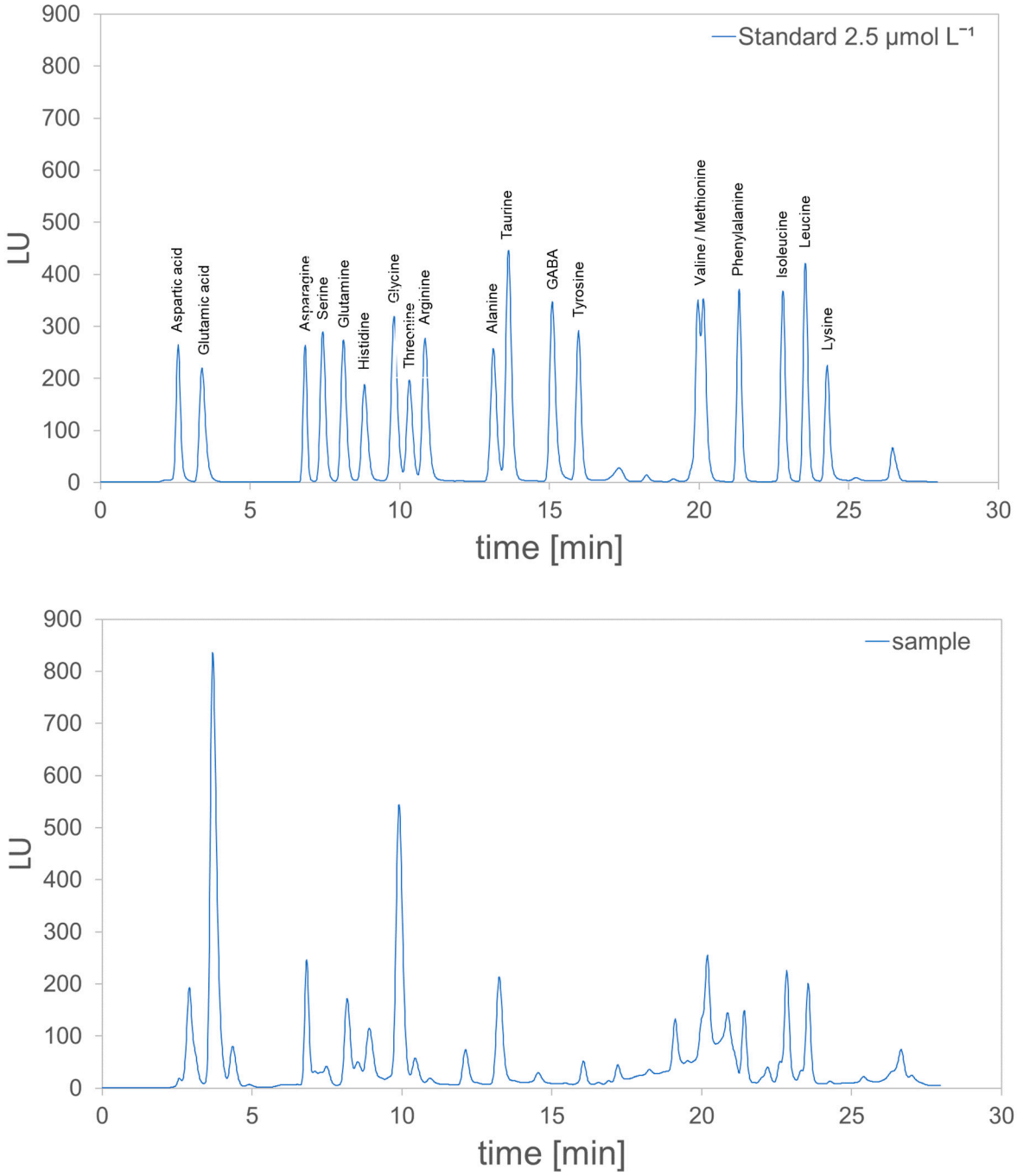


Figure S1. HPLC-Chromatograms of a 2.5 µmol L^{-1} standard (AAS18, Sigma Aldrich; the single amino acid tryptophane (Trp) is missing in that specific standard curve) in MQ-water compared with a sample (undiluted) analysed by the method published in Clifford *et al.*, 2017 [53]. Injection volume 500 µL, gain factor 10, method duration 28 min.

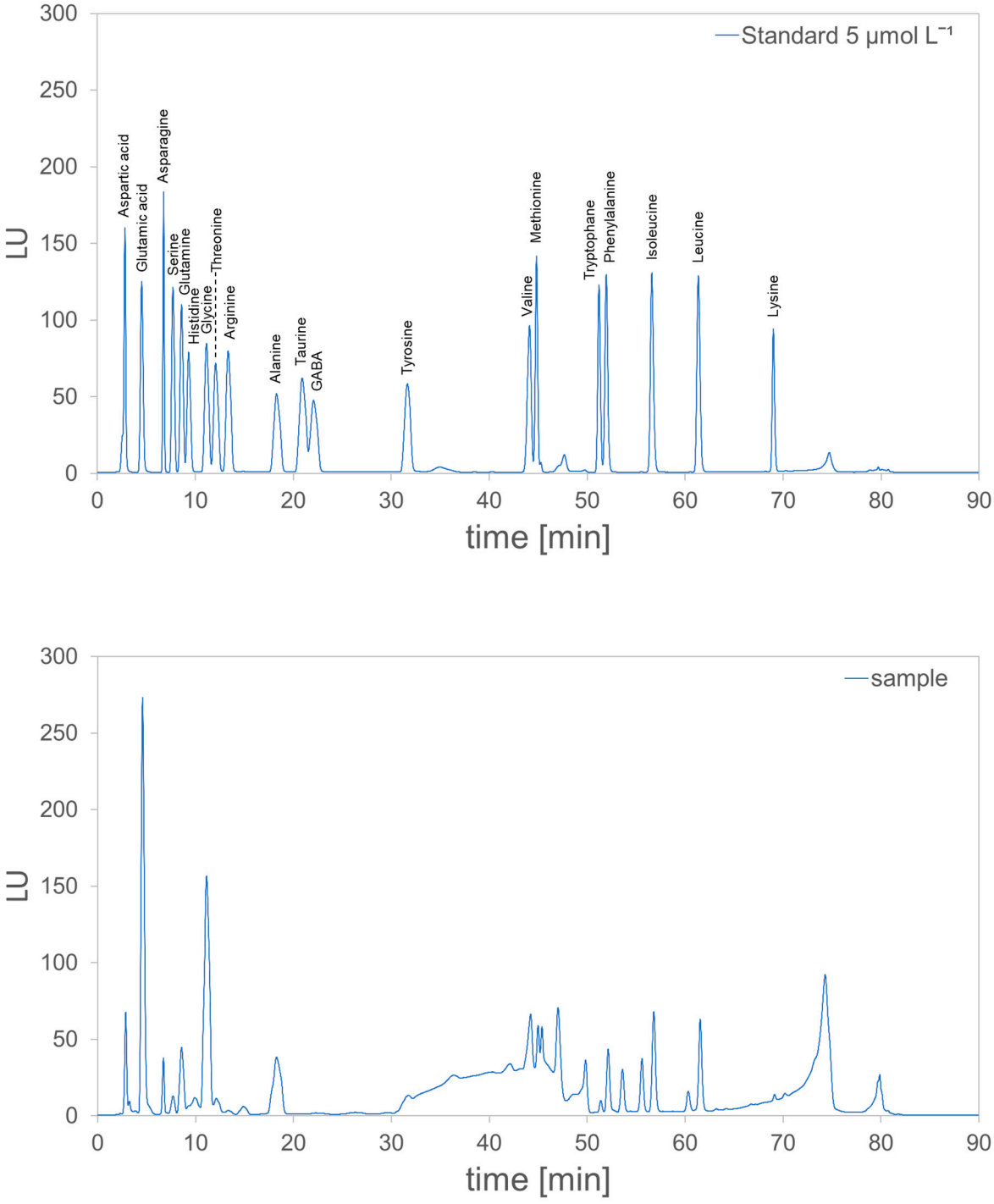


Figure S2. HPLC-Chromatograms of a 5 µmol L^{-1} standard (AAS18, Sigma Aldrich) in MQ-water compared with a sample (dilution 1:4, in MQ-water) analysed by the new method presented in this study. Injection volume 100 µL, gain factor 10, method duration 90 min.

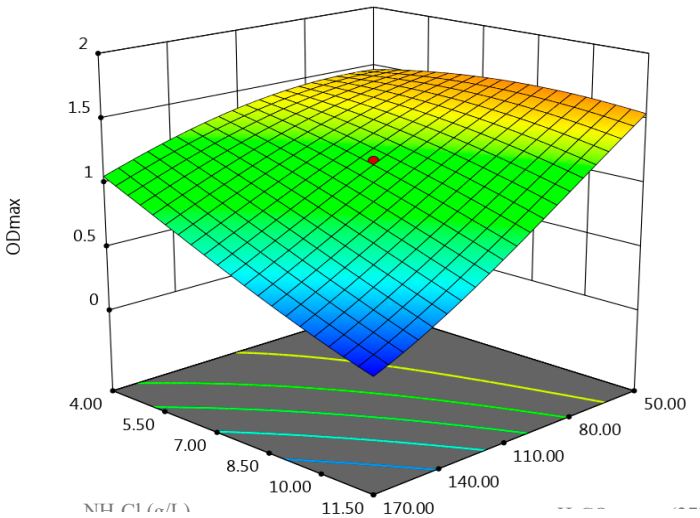


Figure S3. Results of the statistical analysis of the DoE. Significant dependences were calculated for OD_{max}.

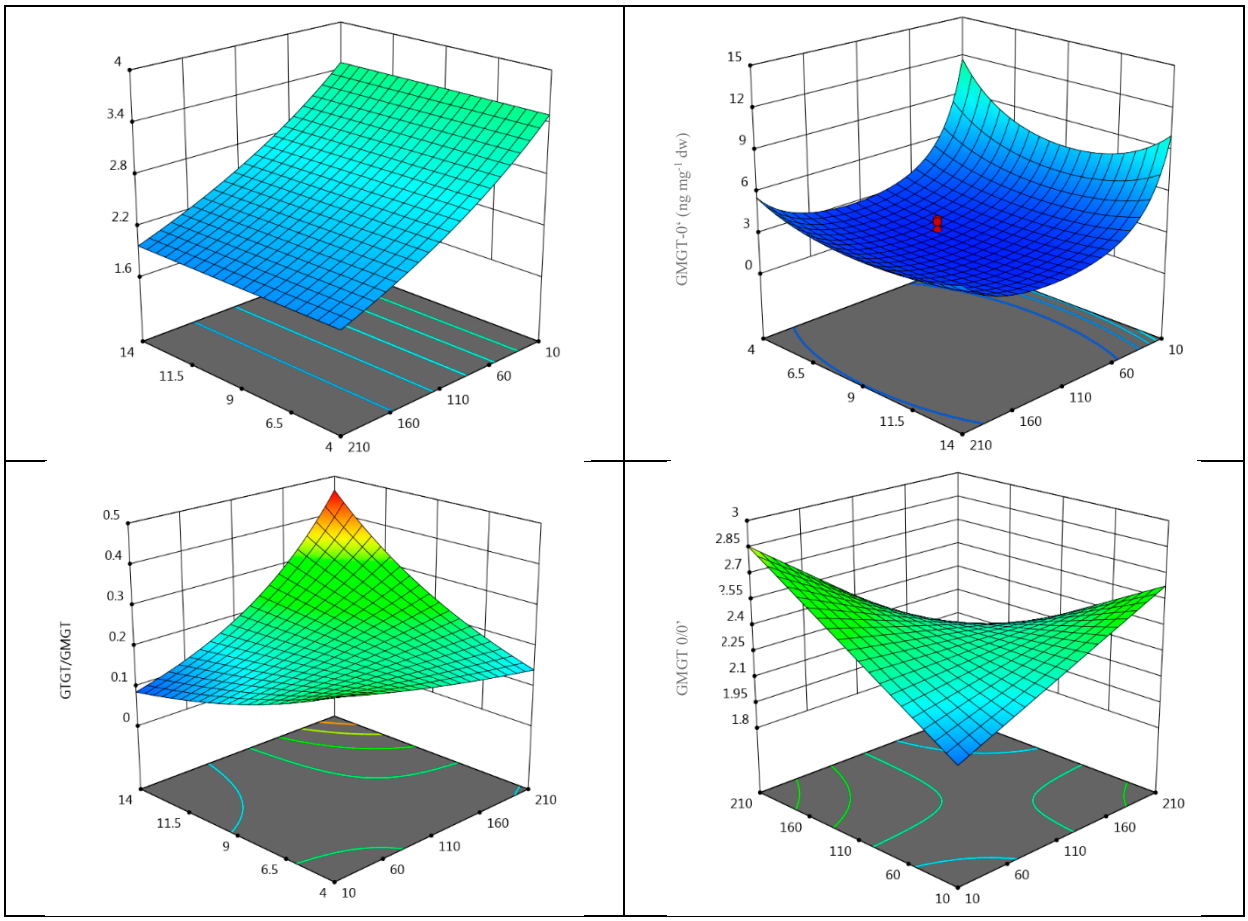
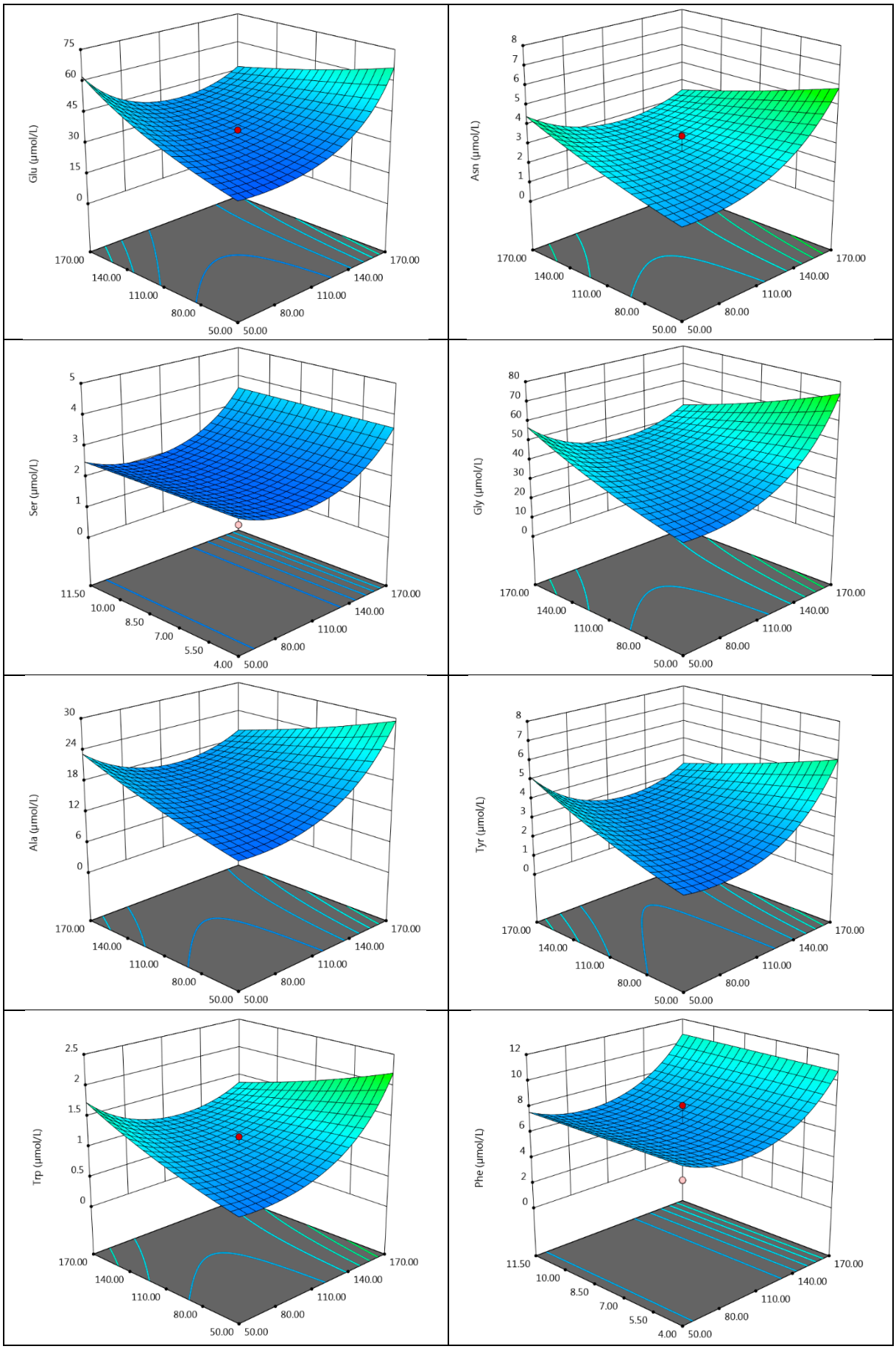


Figure S4. Results of the statistical analysis of the DoE. Significant dependences were calculated for GTGT-0, GMGT-0′, GTGT/GMGT (including both GMGT isomers), and GMGT-0/0′.


Glu (µmol/L)
75
60
45
30
15
0
170.00
140.00
110.00
80.00
50.00
50.00
80.00
110.00
140.00
170.00
Asn (µmol/L)
8
7
6
5
4
3
2
1
0
Ser (µmol/L)
5
4
3
2
1
0
11.50
10.00
8.50
7.00
5.50
4.00
Gly (µmol/L)
80
70
60
50
40
30
20
10
0
Ala (µmol/L)
30
24
18
12
6
0
Tyr (µmol/L)
Trp (µmol/L)
2.5
2
1.5
1
0.5
0
Phe (µmol/L)
12
10
8
6
4
2
0

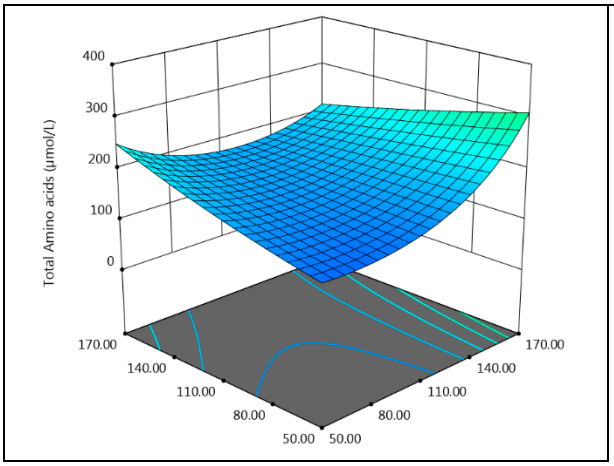


Figure S5. Glu (glutamic acid), Asn (asparagine), Ser (serine), Gly (glycine), Ala (alanine), Tyr (tyrosine), Trp (tryptophan), Phe (phenylalanine), and total amino acids show a dependence on CH_3OH squared.

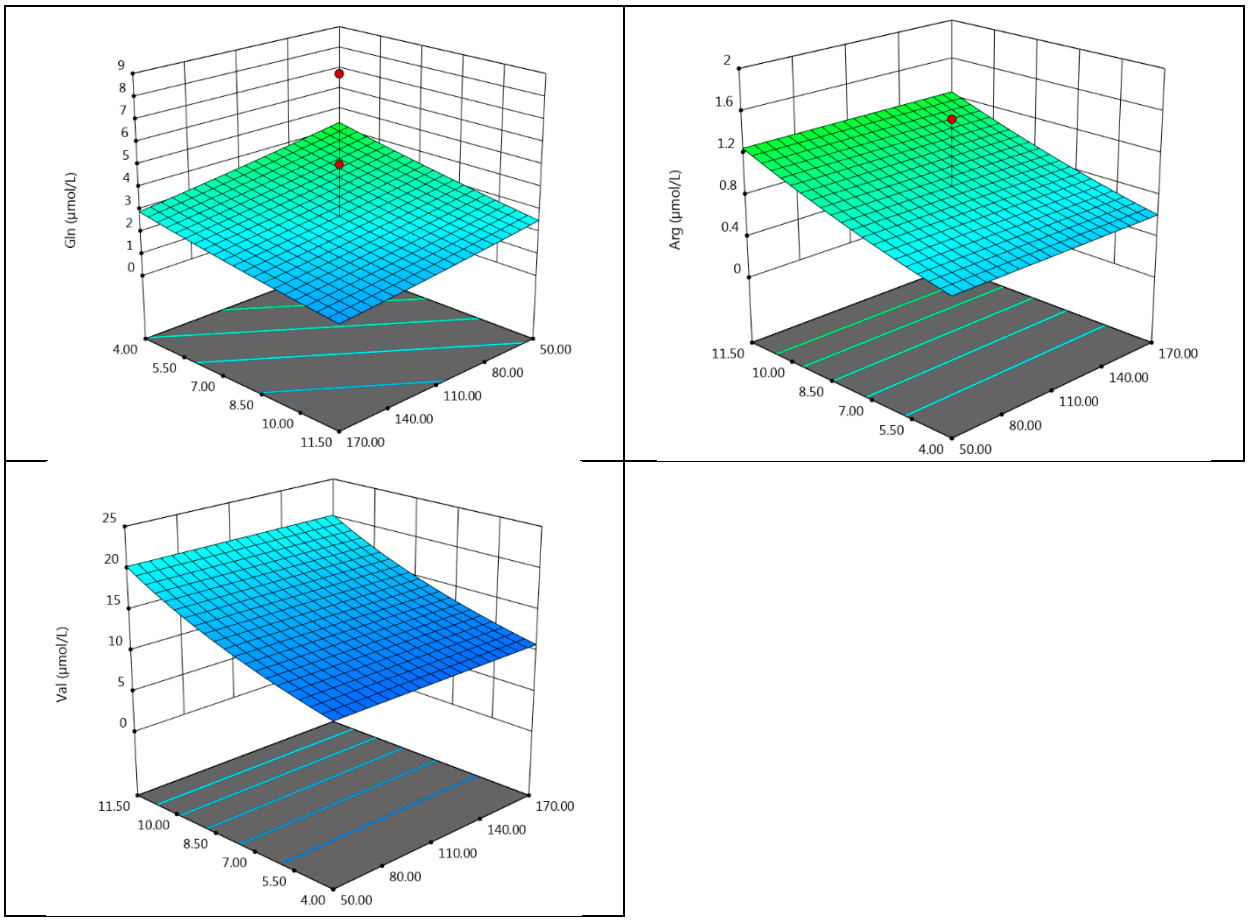


Figure S6. Gln (glutamine), Arg (arginine), and Val (valine) are influenced by the presence of NH_4Cl.

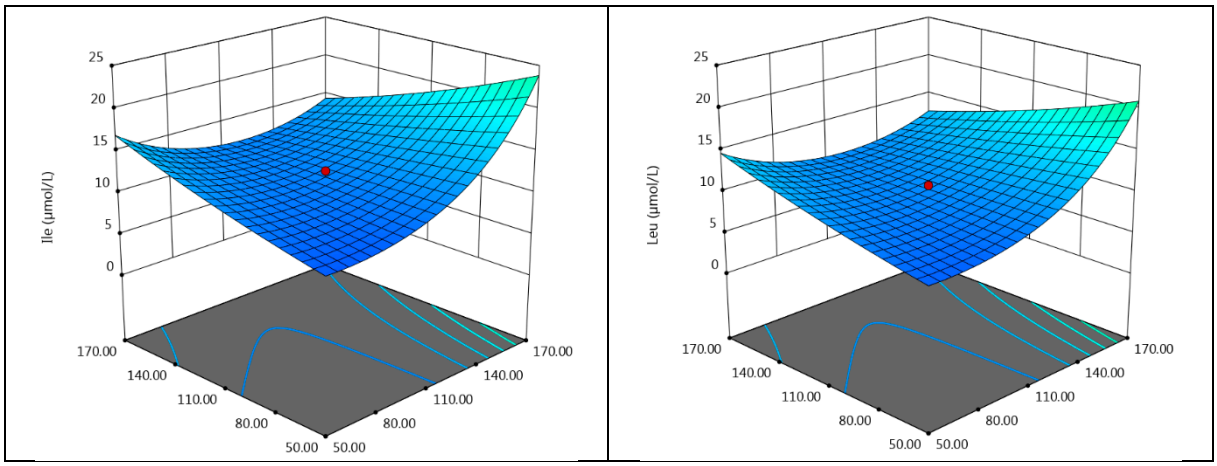


Figure S7. Ile (isoleucine) and Leu (leucine) are characterized by the influence of NH_4Cl and CH_3OH squared.

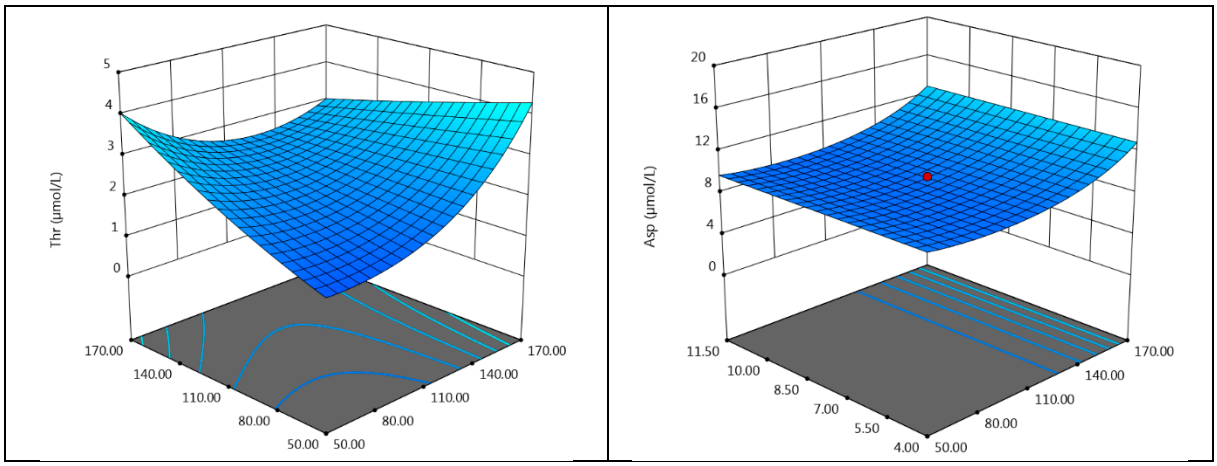


Figure S8. Thr (threonine) is influenced by the interaction of CH_3OH and H_2CO and CH_3OH squared, and Asp (aspartic acid) shows no specific dependency at all.

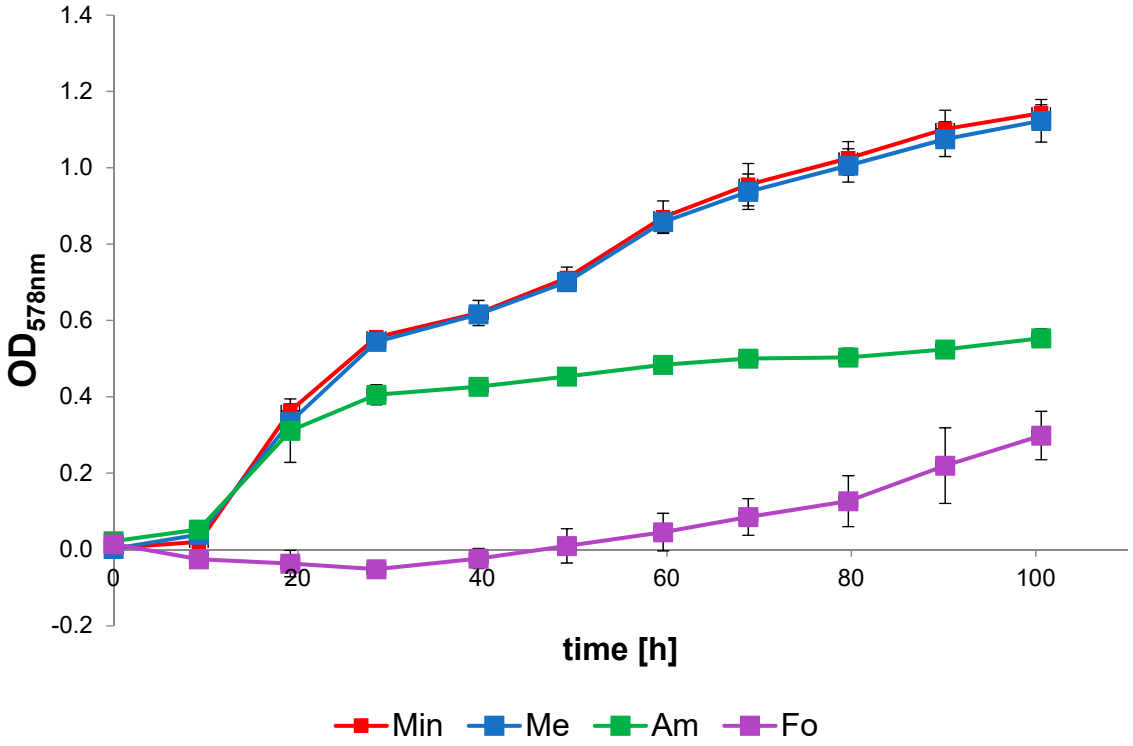


Figure S9. Average growth curves of the four different experiments (n=4, the OD of the respective zero control was subtracted, error bars present respective standard deviations).

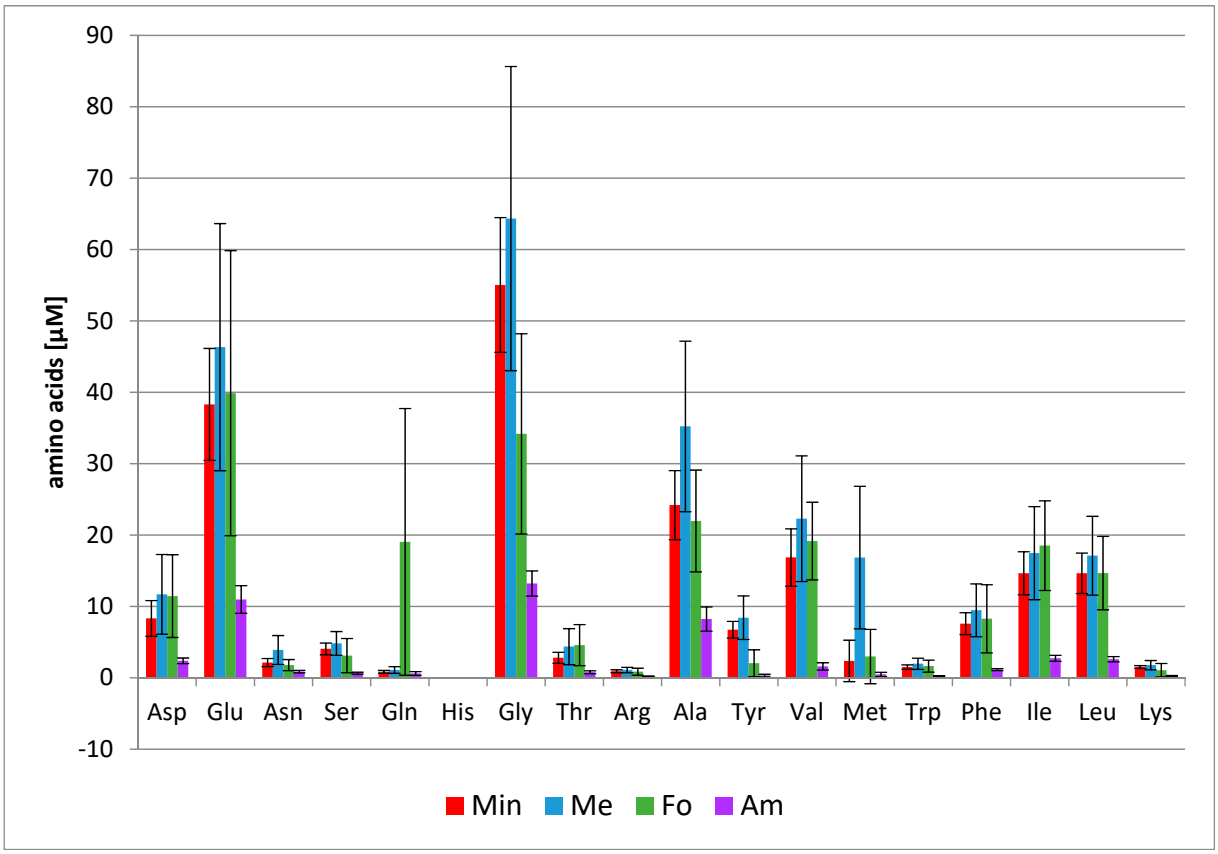


Figure S10. Concentrations of the different amino acids for the four different experimental settings (n=4, error bars present respective standard deviations). The high error of Gln for the "Fo"-setting is caused by a wide spreading of the data points between 4 and 46 µM. However, even the error is high, all single samples of the "Fo"-set showed a value far higher in Gln than in any other sample. Abbreviations of amino acids: Asp = aspartic acid; Glu = glutamic acid; Asn = asparagine; Ser = serine; Gln = glutamine; His = histidine; Gly = glycine; Thr = threonine; Arg = arginine; Ala = alanine; Tyr = tyrosine; Val = valine; Met = methionine; Trp = tryptophan; Phe = phenylalanine; Ile = isoleucine; Leu = leucine; Lys = lysine.