

Optimizing isothiocyanate formation during enzymatic glucosinolate breakdown by adjusting pH value, temperature and dilution in *Brassica* vegetables and *Arabidopsis thaliana*

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Glucosinolates (GLS) in μmol/g fresh weight	<i>Brassica rapa</i> 215		Inactivated <i>Brassica rapa</i> 215		<i>Brassica rapa</i> 374		Inactivated <i>Brassica rapa</i> 374		<i>Arabidopsis thaliana</i> Bur-0		<i>Arabidopsis thaliana</i> Hi-0		<i>Brassica oleracea</i> BroccoCress®		Inactivated <i>Brassica</i> <i>oleracea</i> BroccoCress®		<i>Brassica oleracea</i> white cabbage		Inactivated <i>Brassica</i> <i>oleracea</i> white cabbage		
	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	
2-propenyl (2Prop)	0.000	0.000	0.000	0.000	0.000	0.000	1.806	0.019	5.088	0.003	0.000	0.000	0.000	0.000	1.231	0.018	2.357	0.086			
3-butetyl (3But)	0.296	0.003	0.102	0.001	1.126	0.034	0.676	0.024	2.910	0.011	0.036	0.000	0.020	0.000	0.003	0.001	0.033	0.003	0.048	0.001	
4-pentenyl	0.194	0.006	0.025	0.000	0.391	0.012	0.074	0.003	0.073	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
R-2-OH-3-butenyl	0.053	0.001	0.030	0.002	0.000	0.000	0.000	0.000	0.198	0.005	0.000	0.000	0.011	0.001	0.000	0.000	0.428	0.025	0.701	0.027	
S-2-OH-3-butenyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.355	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
3-(methylthio)propyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.001	0.000	0.000	0.007	0.001	0.000	0.000	0.024	0.001	0.098	0.015	
4-(methylthio)butyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.119	0.045	1.114	0.001	0.014	0.002	0.023	0.001	
3-(methylsulphonyl)propyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.163	0.001	0.151	0.002	0.587	0.012	1.467	0.065	
4-(methylsulphonyl)butyl	0.066	0.025	0.024	0.011	0.010	0.002	0.033	0.010	0.038	0.014	0.000	0.000	5.856	0.078	5.923	0.134	0.240	0.011	0.486	0.021	
7-(methylsulphonyl)heptyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.196	0.001	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
8-(methylsulphonyl)octyl	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.925	0.017	0.477	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
2-phenylethyl	0.012	0.002	0.004	0.000	0.031	0.002	0.011	0.000	0.017	0.004	0.000	0.004	0.019	0.000	0.012	0.000	0.000	0.000	0.000	0.001	0.000
3-indolylmethyl	0.080	0.002	0.034	0.001	0.023	0.002	0.011	0.001	0.252	0.004	0.164	0.004	0.186	0.005	0.165	0.001	0.093	0.004	0.154	0.005	
1-methoxy-3-indolylmethyl	0.005	0.002	0.003	0.000	0.028	0.002	0.010	0.001	0.044	0.000	0.042	0.001	0.047	0.001	0.052	0.003	0.016	0.000	0.023	0.001	
4-hydroxy-3-indolylmethyl	0.006	0.000	0.003	0.001	0.004	0.001	0.003	0.000	0.007	0.001	0.005	0.001	0.147	0.001	0.121	0.001	0.112	0.008	0.194	0.013	
4-methoxy-3-indolylmethyl	0.021	0.001	0.015	0.000	0.014	0.000	0.006	0.000	0.065	0.004	0.026	0.000	0.113	0.003	0.102	0.001	0.012	0.001	0.007	0.000	
Total GLS	0.666	0.011	0.240	0.011	1.627	0.054	0.824	0.015	6.933	0.041	5.872	0.007	7.687	0.020	7.643	0.135	2.790	0.024	5.559	0.235	

Influence of temperature and pH value

$\mu\text{mol/g}$ fresh weight

	4°C, pH 4.2	4°C, pH 6.3	4°C, pH 8.3	4	22°C, pH 4.2	22°C, pH 6.3	22°C, pH 8.3	37°C, pH 4.2	37°C, pH 6.3	37°C, pH 8.3
<i>Brassica rapa</i> 215										
3-but enyl CN	0.0185 ± 0.0093	0.0034 ± 0.0010	0.0097 ± 0.0029		0.0064 ± 0.0055	0.0024 ± 0.0013	0.0017 ± 0.0019	0.0105 ± 0.0084	0.0130 ± 0.0206	0.0061 ± 0.0064
3-but enyl ITC	0.0223 ± 0.0019	0.0067 ± 0.0043	0.0162 ± 0.0014		0.0076 ± 0.0050	0.0056 ± 0.0028	0.0146 ± 0.0038	0.0020 ± 0.0006	0.0000 ± 0.0000	0.0104 ± 0.0008
1-cyano-3,4-epithiobutane	0.0052 ± 0.0011	0.0687 ± 0.0202	0.0507 ± 0.0051		0.0101 ± 0.0117	0.0571 ± 0.0210	0.0241 ± 0.0151	0.0081 ± 0.0037	0.0182 ± 0.0026	0.0200 ± 0.0042
4-pentenyl ITC	0.0506 ± 0.0135	0.0015 ± 0.0017	0.0032 ± 0.0005		0.0294 ± 0.0477	0.0020 ± 0.0006	0.0032 ± 0.0006	0.0000 ± 0.0000	0.0006 ± 0.0010	0.0015 ± 0.0007
1-cyano-4,5-epithiopentane	0.0032 ± 0.0004	0.0156 ± 0.0045	0.0066 ± 0.0005		0.0031 ± 0.0027	0.0095 ± 0.0023	0.0047 ± 0.0006	0.0011 ± 0.0019	0.0038 ± 0.0013	0.0037 ± 0.0007
2-phenylethyl CN	0.0081 ± 0.0030	0.0040 ± 0.0017	0.0017 ± 0.0004		0.0053 ± 0.0055	0.0034 ± 0.0014	0.0016 ± 0.0003	0.0013 ± 0.0001	0.0013 ± 0.0003	0.0015 ± 0.0003
2-phenylethyl ITC	0.0024 ± 0.0006	0.0011 ± 0.0001	0.0014 ± 0.0012		0.0014 ± 0.0005	0.0015 ± 0.0003	0.0008 ± 0.0001	0.0014 ± 0.0002	0.0018 ± 0.0004	0.0004 ± 0.0002
<i>Brassica rapa</i> 374										
3-but enyl CN	0.1030 ± 0.0575	0.0697 ± 0.0071	0.0477 ± 0.0141		0.0568 ± 0.0437	0.1249 ± 0.0558	0.0808 ± 0.0447	0.0889 ± 0.0503	0.1762 ± 0.0913	0.0690 ± 0.0662
3-but enyl ITC	0.3106 ± 0.0869	0.5477 ± 0.1482	0.6467 ± 0.2295		0.3029 ± 0.2533	0.4026 ± 0.2416	0.6564 ± 0.2298	0.6754 ± 0.5143	0.4092 ± 0.1777	0.3547 ± 0.3055
1-cyano-3,4-epithiobutane	1.6773 ± 1.2678	0.9409 ± 0.1564	0.7627 ± 0.3608		1.0321 ± 1.1254	1.4932 ± 1.0723	0.8237 ± 0.3709	0.9370 ± 0.2860	1.7951 ± 0.8852	0.6017 ± 0.5355
4-pentenyl ITC	0.0679 ± 0.0691	0.0577 ± 0.0345	0.1572 ± 0.0748		0.0727 ± 0.1101	0.0587 ± 0.0736	0.0660 ± 0.0281	0.0779 ± 0.0182	0.1113 ± 0.1012	0.0662 ± 0.0604
1-cyano-4,5-epithiopentane	0.2010 ± 0.1791	0.0601 ± 0.0424	0.1380 ± 0.1081		0.2040 ± 0.3283	0.3083 ± 0.4979	0.0710 ± 0.0453	0.0724 ± 0.0325	0.3807 ± 0.5503	0.0443 ± 0.0451
2-phenylethyl CN	0.0235 ± 0.0202	0.0156 ± 0.0128	0.0104 ± 0.0074		0.0242 ± 0.0306	0.0272 ± 0.0209	0.0227 ± 0.0168	0.0237 ± 0.0217	0.0256 ± 0.0102	0.0072 ± 0.0063
2-phenylethyl ITC	0.0140 ± 0.0076	0.0193 ± 0.0110	0.0106 ± 0.0036		0.0117 ± 0.0058	0.0221 ± 0.0156	0.0281 ± 0.0210	0.0314 ± 0.0239	0.0221 ± 0.0052	0.0086 ± 0.0075
<i>Arabidopsis thaliana</i> Bur-0										
2-propenyl CN	0.0248 ± 0.0090	0.0124 ± 0.0060	0.0115 ± 0.0054		0.0240 ± 0.0082	0.0150 ± 0.0010	0.0119 ± 0.0057	0.0410 ± 0.0115	0.0128 ± 0.0038	0.0199 ± 0.0070
2-propenyl ITC	0.0183 ± 0.0038	0.0272 ± 0.0092	0.0242 ± 0.0081		0.0222 ± 0.0074	0.0367 ± 0.0076	0.0317 ± 0.0177	0.0572 ± 0.0079	0.0179 ± 0.0046	0.0332 ± 0.0088
1-cyano-2,3-epithiopropane	0.5712 ± 0.0850	0.4891 ± 0.1957	0.4426 ± 0.2189		0.5002 ± 0.1549	0.5435 ± 0.0668	0.3834 ± 0.1630	0.3367 ± 0.1658	0.3126 ± 0.0868	0.4535 ± 0.1535
3-but enyl CN	0.1044 ± 0.0450	0.0415 ± 0.0429	0.0393 ± 0.0422		0.0987 ± 0.0386	0.0842 ± 0.0114	0.0461 ± 0.0309	0.1295 ± 0.0421	0.0659 ± 0.0121	0.0876 ± 0.0258
3-but enyl ITC	0.0263 ± 0.0068	0.0435 ± 0.0227	0.0369 ± 0.0185		0.0271 ± 0.0129	0.0557 ± 0.0183	0.0438 ± 0.0276	0.0668 ± 0.0194	0.0165 ± 0.0049	0.0381 ± 0.0161
1-cyano-3,4-epithiobutane	0.9930 ± 0.3247	0.9883 ± 0.4846	0.8514 ± 0.5239		1.0288 ± 0.4774	1.0590 ± 0.2590	0.7077 ± 0.3108	0.6644 ± 0.3814	0.4646 ± 0.1398	0.8601 ± 0.3931
2-hydroxy-3-but enyl CN	0.0139 ± 0.0027	0.0136 ± 0.0040	0.0116 ± 0.0064		0.0168 ± 0.0023	0.0174 ± 0.0027	0.0120 ± 0.0029	0.0186 ± 0.0009	0.0161 ± 0.0053	0.0147 ± 0.0017
1-cyano-2-hydroxy-3,4-epithiobutane	0.1319 ± 0.0256	0.1441 ± 0.0406	0.1110 ± 0.0640		0.1423 ± 0.0226	0.1592 ± 0.0228	0.0990 ± 0.0249	0.0960 ± 0.0284	0.1152 ± 0.0403	0.1155 ± 0.0102
4-pentenyl ITC	0.0000 ± 0.0000	0.0007 ± 0.0012	0.0007 ± 0.0012		0.0013 ± 0.0012	0.0013 ± 0.0011	0.0004 ± 0.0007	0.0010 ± 0.0009	0.0000 ± 0.0000	0.0007 ± 0.0006
1-cyano-4,5-epithiopentane	0.0297 ± 0.0049	0.0267 ± 0.0161	0.0212 ± 0.0171		0.0300 ± 0.0182	0.0279 ± 0.0121	0.0159 ± 0.0057	0.0146 ± 0.0088	0.0112 ± 0.0049	0.0198 ± 0.0104
2-phenylethyl CN	0.0113 ± 0.0011	0.0113 ± 0.0025	0.0102 ± 0.0034		0.0112 ± 0.0027	0.0112 ± 0.0013	0.0096 ± 0.0013	0.0083 ± 0.0019	0.0082 ± 0.0012	0.0111 ± 0.0013
2-phenylethyl ITC	0.0017 ± 0.0004	0.0011 ± 0.0001	0.0009 ± 0.0001		0.0009 ± 0.0007	0.0012 ± 0.0003	0.0011 ± 0.0001	0.0009 ± 0.0003	0.0023 ± 0.0004	0.0017 ± 0.0002
5-(methylthio)pentyl CN	0.0020 ± 0.0004	0.0020 ± 0.0006	0.0015 ± 0.0008		0.0023 ± 0.0006	0.0023 ± 0.0004	0.0012 ± 0.0004	0.0013 ± 0.0006	0.0012 ± 0.0003	0.0016 ± 0.0002
6-(methylthio)hexyl CN	0.0046 ± 0.0009	0.0034 ± 0.0014	0.0030 ± 0.0018		0.0036 ± 0.0015	0.0033 ± 0.0002	0.0023 ± 0.0007	0.0018 ± 0.0010	0.0014 ± 0.0005	0.0030 ± 0.0011
7-(methylthio)heptyl CN	0.0217 ± 0.0022	0.0176 ± 0.0064	0.0175 ± 0.0087		0.0177 ± 0.0043	0.0164 ± 0.0018	0.0129 ± 0.0040	0.0085 ± 0.0052	0.0062 ± 0.0030	0.0152 ± 0.0038
7-(methylthio)heptyl ITC	0.0017 ± 0.0007	0.0024 ± 0.0034	0.0035 ± 0.0021		0.0031 ± 0.0032	0.0055 ± 0.0017	0.0027 ± 0.0027	0.0039 ± 0.0024	0.0058 ± 0.0001	0.0026 ± 0.0031
8-(methylthio)octyl CN	0.0413 ± 0.0399	0.0276 ± 0.0110	0.0335 ± 0.0143		0.0230 ± 0.0077	0.0220 ± 0.0034	0.0187 ± 0.0059	0.0106 ± 0.0080	0.0080 ± 0.0042	0.0237 ± 0.0065
8-(methylthio)octyl ITC	0.0058 ± 0.0006	0.0013 ± 0.0012	0.0020 ± 0.0010		0.0032 ± 0.0024	0.0010 ± 0.0001	0.0008 ± 0.0008	0.0068 ± 0.0050	0.0007 ± 0.0007	0.0020 ± 0.0016
8-(methylsulphinyl)octyl CN	0.0170 ± 0.0081	0.0154 ± 0.0088	0.0117 ± 0.0055		0.0116 ± 0.0057	0.0175 ± 0.0031	0.0143 ± 0.0096	0.0084 ± 0.0045	0.0106 ± 0.0064	0.0174 ± 0.0106
indole-3-acetonitrile	0.0138 ± 0.0014	0.0175 ± 0.0036	0.0125 ± 0.0040		0.0076 ± 0.0010	0.0174 ± 0.0007	0.0115 ± 0.0016	0.0029 ± 0.0016	0.0121 ± 0.0051	0.0140 ± 0.0023
<i>Arabidopsis thaliana</i> Hi-0										
2-propenyl CN	0.2353 ± 0.0785	0.4047 ± 0.2263	0.2097 ± 0.0463		0.1713 ± 0.0961	0.3074 ± 0.0568	0.1600 ± 0.0186	0.1952 ± 0.0610	0.1445 ± 0.0464	0.0848 ± 0.0112
2-propenyl ITC	0.9009 ± 0.2021	0.5600 ± 0.1739	0.8381 ± 0.0522		0.6106 ± 0.1781	0.6050 ± 0.1447	0.8405 ± 0.2327	0.5789 ± 0.1284	0.3421 ± 0.0246	0.6884 ± 0.1199
1-cyano-2,3-epithiopropane	0.0044 ± 0.0008	0.0031 ± 0.0018	0.0015 ± 0.0007		0.0056 ± 0.0013	0.0026 ± 0.0010	0.0010 ± 0.0001	0.0105 ± 0.0031	0.0010 ± 0.0005	0.0005 ± 0.0002
3-but enyl ITC	0.0036 ± 0.0011	0.0025 ± 0.0013	0.0034 ± 0.0008		0.0029 ± 0.0016	0.0028 ± 0.0009	0.0033 ± 0.0014	0.0037 ± 0.0007	0.0016 ± 0.0001	0.0034 ± 0.0011
7-(methylthio)heptyl ITC	0.0013 ± 0.0002	0.0037 ± 0.0025	0.0011 ± 0.0002		0.0014 ± 0.0005	0.0067 ± 0.0010	0.0010 ± 0.0003	0.0014 ± 0.0003	0.0063 ± 0.0008	0.0053 ± 0.0004
8-(methylthio)octyl CN	0.0007 ± 0.0007	0.0007 ± 0.0008	0.0007 ± 0.0007		0.0012 ± 0.0006	0.0011 ± 0.0001	0.0013 ± 0.0001	0.0013 ± 0.0004	0.0020 ± 0.0003	0.0014 ± 0.0002
8-(methylthio)octyl ITC	0.0123 ± 0.0020	0.0055 ± 0.0023	0.0082 ± 0.0021		0.0104 ± 0.0039	0.0042 ± 0.0007	0.0062 ± 0.0024	0.0112 ± 0.0024	0.0019 ± 0.0004	0.0033 ± 0.0011
2-phenylethyl CN	0.0010 ± 0.0003	0.0010 ± 0.0003	0.0008 ± 0.0001		0.0013 ± 0.0001	0.0010 ± 0.0001	0.0012 ± 0.0003	0.0012 ± 0.0003	0.0009 ± 0.0007	0.0011 ± 0.0001
indole-3-acetonitrile	0.0010 ± 0.0001	0.0022 ± 0.0006	0.0009 ± 0.0009		0.0008 ± 0.0003	0.0007 ± 0.0006	0.0003 ± 0.0005	0.0000 ± 0.0000	0.0005 ± 0.0004	0.0006 ± 0.0006
<i>Brassica oleracea</i> BroccoCress®										
4°C, pH 3.8	1.0418 ± 0.1780	0.9735 ± 0.1604	0.8010 ± 0.2794		0.9300 ± 0.0330	0.9218 ± 0.1484	0.7404 ± 0.1278	0.2935 ± 0.0492	1.0183 ± 0.2372	0.4334 ± 0.0684
4-(methylthio)butyl CN	0.2701 ± 0.2914	0.1013 ± 0.0382	0.5326 ± 0.0730		0.3022 ± 0.0991	0.1301 ± 0.0273	0.4201 ± 0.2004	0.3974 ± 0.0771	0.0760 ± 0.0499	0.2453 ± 0.0916
5-(methylthio)pentyl CN	0.0008 ± 0.0002	0.0042 ± 0.0009	0.0032 ± 0.0013		0.0039 ± 0.0002	0.0042 ± 0.0010	0.0031 ± 0.0008	0.0016 ± 0.0002	0.0021 ± 0.0009	0.0016 ± 0.0004
3-(methylsulphinyl)propyl CN	0.0618 ± 0.0496	0.0710 ± 0.1009	0.0179 ± 0.0047		0.0280 ± 0.0073	0.0290 ± 0.0118	0.0191 ± 0.0061	0.0126 ± 0.0052	0.0286 ± 0.0116	0.0177 ± 0.0129
3-(methylsulphinyl)propyl ITC	0.0194 ± 0.0140	0.0125 ± 0.0064	0.0377 ± 0.0066		0.0285 ± 0.0050	0.0183 ± 0.0041	0.0519 ± 0.0138	0.0734 ± 0.0104	0.0367 ± 0.0490	0.1544 ± 0.0962
4-(methylsulphinyl)butyl CN	2.5013 ± 0.7334	1.9967 ± 0.2595	1.6946 ± 0.2707		2.1454 ± 0.4056	1.9604 ± 0.3395	1.0231 ± 0.2523	0.6481 ± 0.0413	1.2559 ± 0.2341	0.5151 ± 0.0578
4-(methylsulphinyl)butyl ITC	1.3149 ± 1.5635	0.4885 ± 0.1468	2.6549 ± 0.1353		1.6530 ± 0.3352	0.6452 ± 0.1580	2.0664 ± 0.5858	2.6445 ± 0.2629	0.8798 ± 0.8123	2.8103 ± 0.9438
2-phenylethyl CN	0.0287 ± 0.0043	0.0371 ± 0.0043	0.0230 ± 0.0041		0.0377 ± 0.0034	0.0456 ± 0.0099	0.0282 ± 0.0059	0.0283 ± 0.0014	0.0391 ± 0.0088	0.0222 ± 0.0036
2-phenylethyl ITC	0.0088 ± 0.0096	0.0062 ± 0.0020	0.0168 ± 0.0047		0.0105 ± 0.0010	0.0080 ± 0.0040	0.0153 ± 0.0037	0.0128 ± 0.0017	0.0040 ± 0.0039	0.0058 ± 0.0042
indole-3-acetonitrile	0.0511 ± 0.0184	0.0677 ± 0.0179	0.0153 ± 0.0046		0.0406 ± 0.0045	0.0542 ± 0.0059	0.0181 ± 0.0064	0.0187 ± 0.0036	0.0493 ± 0.0190	0.0159 ± 0.0040
4-m										

Influence of temperature at pH 4.2 on GLS-degradation in Bur-0

pH 4.2	% of 2-propenyl glucosinolate breakdowns			% of 3-butenyl glucosinolate breakdowns		
T	2-propenyl CN	2-propenyl ITC	1-cyano-2,3-epithiopropane	3-butenyl CN	3-butenyl ITC	1-cyano-3,4-epithiobutane
4	2.99 ± 0.60 a	3.96 ± 0.81 a	93.05 ± 1.09 b	2.62 ± 1.32 a	9.28 ± 2.12 a	88.10 ± 2.36 b
22	4.20 ± 1.45 a	4.35 ± 0.39 a	91.45 ± 1.66 b	2.42 ± 0.95 a	8.74 ± 0.66 a	88.84 ± 1.15 b
37	14.42 ± 4.60 b	9.81 ± 1.47 b	75.77 ± 6.03 a	8.56 ± 2.48 b	16.21 ± 3.44 b	75.23 ± 5.89 a

Influence of dilution μmol/g fresh weight

Glucosinolate hydrolysis products in myrosinase inactivated plant material ($\mu\text{mol/g FW}$)

Brassica rapa 215

3-but-enyl ITC 0.0083 ± 0.0081

Brassica rapa 374

3-but-enyl ITC 0.0024 ± 0.0007

Brassica oleracea BroccoCress®

4-(methylthio)butyl CN 0.0771 ± 0.0135

4-(methylthio)butyl ITC 0.0048 ± 0.0012

4-(methylsulfinyl)butyl CN 0.1747 ± 0.0150

2-phenylethyl CN 0.0095 ± 0.0004

indole-3-acetonitrile 0.0095 ± 0.0019

4-methoxyindole-3-acetonitrile 0.0054 ± 0.0013

Brassica oleracea white cabbage sprouts

2-propenyl CN 0.0128 ± 0.0014

2-propenyl ITC 0.0014 ± 0.0024

2-hydroxy-3-but enyl CN 0.0436 ± 0.0012

3-(methylthio)propyl CN 0.0065 ± 0.0001

4-(methylthio)butyl CN 0.0022 ± 0.0001

4-(methylthio)butyl ITC 0.0004 ± 0.0007

