

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	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-butenyl (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-(methylsulphinyl)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-(methylsulphinyl)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-(methylsulphinyl)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-(methylsulphinyl)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	0.001	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 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-epithiopropene	3-butenyl CN	3-butenyl ITC
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



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

*Brassica rapa* 215

3-butenyl ITC 0.0083  $\pm$  0.0081

*Brassica rapa* 374

3-butenyl ITC 0.0024  $\pm$  0.0007

*Brassica oleracea* BroccoCress<sup>®</sup>

4-(methylthio)butyl CN 0.0771  $\pm$  0.0135

4-(methylthio)butyl ITC 0.0048  $\pm$  0.0012

4-(methylsulfinyl)butyl CN 0.1747  $\pm$  0.0150

2-phenylethyl CN 0.0095  $\pm$  0.0004

indole-3-acetonitrile 0.0095  $\pm$  0.0019

4-methoxyindole-3-acetonitrile 0.0054  $\pm$  0.0013

*Brassica oleracea* white cabbage sprouts

2-propenyl CN 0.0128  $\pm$  0.0014

2-propenyl ITC 0.0014  $\pm$  0.0024

2-hydroxy-3-butenyl CN 0.0436  $\pm$  0.0012

3-(methylthio)propyl CN 0.0065  $\pm$  0.0001

4-(methylthio)butyl CN 0.0022  $\pm$  0.0001

4-(methylthio)butyl ITC 0.0004  $\pm$  0.0007

