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Hydroxylated phenylacetamides derived from bioactive benzoxazinoids are bioavailable in humans after habitual consumption of whole grain sourdough rye bread

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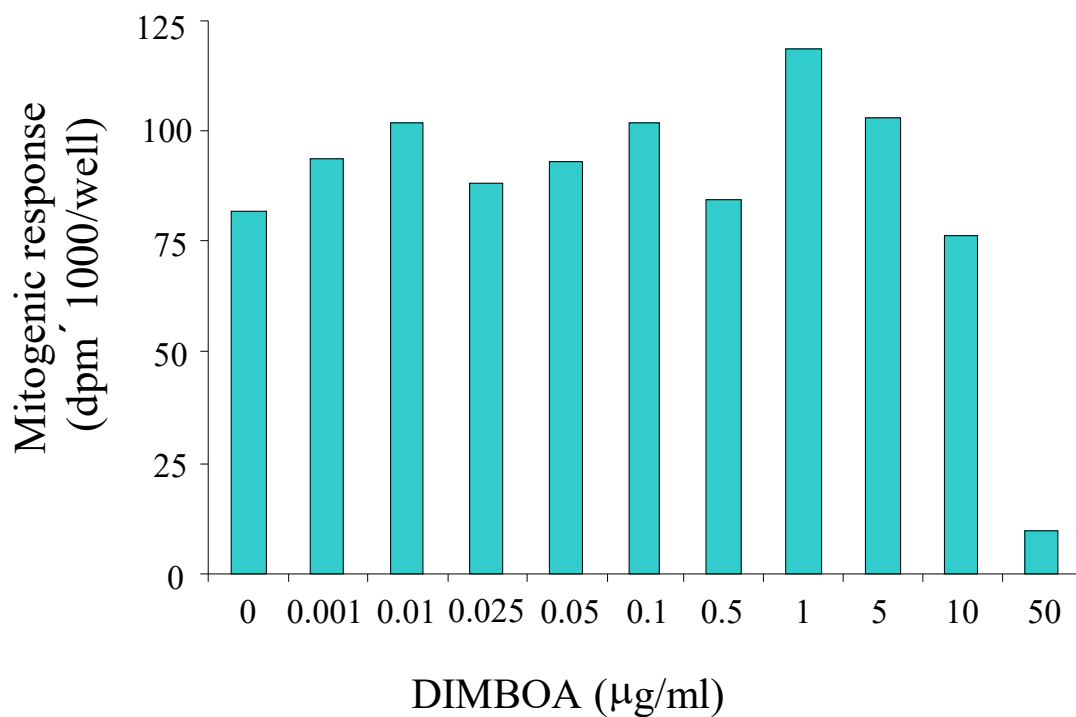
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Supporting Information Data S1. Effects of DIMBOA on proliferation of primary mammary epithelial cells in collagen gel cultures *



Data redrawn from: Brandt, K., Christensen, L.P., Hansen, S.L., Young, J.F., *et al.*, Functions of bioactive plant compounds and effect on humans. *Innov. Food Tech.* 2001, 10, 60-62.

Supporting Information Table S1. The whole grain rye foods included in the study

Serving of rye wholegrain food Serving Size Portion weight (g) Average wholegrain content per portion (g)

Rye bread	1 slice	25	12
Rye muesli	1 packet	28	17
Rye porridge	1 packet	19	16
Rye pasta	1 packet	90	18

Each serving of WG food approximated 16g of whole grains to mimic the serving size advocated by the USDA in its Dietary Guidelines for Americans (USDA, 2005). All food items are declared as 'fresh' matter weight (as obtained at the point of purchase; before milk added to breakfast cereals or boiled in the case of pasta)".

Supporting Information Table S2. Study participant biodata together with the FFQ consumption scores of rye products consumed and plasma alkylresorcinol levels during the three dietary intervention phases of the GrainMark study.

Participant Code	Sex	Age	BMI (at study entry)	Total rye foods ^a			Rye bread			Rye flakes ^b			Rye pasta			Total AR ^f (nmol/l)		
				Wash out ^c	Dose 1 ^d	Dose 2 ^e	Washout ^c	Dose 1 ^d	Dose 2 ^e	Washout ^c	Dose 1 ^d	Dose 2 ^e	Washout ^c	Dose 1 ^d	Dose 2 ^e	Washout ^c	Dose 1 ^d	Dose 2 ^e
101	F	58	27.41	0	3.29	2.01	0	0.79	0.79	0	2.5	1.22	0	0	0	25.4	105.1	163.5
102	F	56	24.62	0.86	0.86	3.72	0	0	2.5	0.86	0.86	1.22	0	0	0	33.1	135.0	268.1
108	M	47	30.22	0	1.43	3.29	0	0.43	0.79	0	1	2.5	0	0	0	21.4	53.7	159.4
114	M	61	29.29	0	0	7	0	0	2.5	0	0	4.5	0	0	0	27.1	152.7	209.2
115	F	55	22.61	0	3.43	3.5	0	2.5	2.5	0	0.79	1	0	0.14	0	14.8	83.7	85.5
119	M	52	23.87	0	1.22	3.5	0	0	2.5	0	1.22	1	0	0	0	69.8	289.6	287.4
120	F	51	25.27	0	0.43	4.08	0	0.43	2.5	0	0	1.58	0	0	0	17.4	75.0	217.0
122	F	61	24.46	0	2.36	5.36	0	1	4.5	0	1.22	0.86	0	0.14	0	15.8	99.1	148.3
123	M	66	32.02	0	2.14	5.5	0	1	4.5	0	1	1	0	0.14	0	21.0	97.5	177.2
125	F	54	25.23	0	2.01	3.93	0	0.79	2.5	0	1.22	1.43	0	0	0	23.1	157.0	151.0
126	F	63	21.51	0	0.79	6	0	0.79	2.5	0	0	3.5	0	0	0	19.7	417.5	890.7
127	M	51	32.31	0	1.58	4.08	0	0.79	2.5	0	0.79	1.58	0	0	0	19.6	104.3	244.2
130	F	55	24.34	0	0.79	2.5	0	0.79	2.5	0	0	0	0	0	0	35.3	204.5	299.8
136	F	47	23.85	0	1.43	2.58	0	0.43	0.79	0	0.86	1.79	0	0.14	0	23.7	111.7	184.0
139	M	52	26.76	0	2	2.14	0	0.43	0	0	1.14	2	0	0.43	0.14	22.9	133.8	280.0
140	M	56	30.72	0	1.65	0	0	0.43	0	0	0.79	0	0	0.43	0	20.2	119.1	328.0
142	F	60	23.42	0	2.08	3.64	0	0.79	2.5	0	0.86	1	0	0.43	0.14	29.4	128.7	418.7
143	F	49	22.28	0	1.43	3.64	0	0	2.5	0	1	1	0	0.43	0.14	32.9	85.4	190.6
151	M	46	25.25	0	1.72	2.79	0	0.43	0.79	0	0.86	2	0	0.43	0	27.1	123.1	287.2
152	F	49	22.86	0	0.57	3.5	0	0.43	2.5	0	0	1	0	0.14	0	16.4	70.3	194.9
155	M	62	31.79	0	5.79	2.79	0	0.79	0.79	0	5	2	0	0	0	23.2	82.6	136.5
156	M	47	23.44	0	1	6.22	0	0	2.5	0	0.57	3.29	0	0.43	0.43	22.3	90.2	229.9
157	M	55	27.38	0	1.5	3.64	0	0.79	2.5	0	0.57	1	0	0.14	0.14	21.5	145.0	220.9
158	F	61	27.24	0	0.57	5.29	0	0	2.5	0	0.43	1.79	0	0.14	1	23.5	59.2	20.7
168	M	52	30.31	0	1.86	3.72	0	0.79	0.43	0	0.93	3.29	0	0.14	0	30.5	363.0	623.0
170	M	53	29.52	0	2.93	5.14	0	0.43	2.5	0	2.5	2.5	0	0	0.14	53.9	130.8	317.4
172	F	46	22.13	0	1.65	3.36	0	0.79	2.5	0	0.43	0.43	0	0.43	0.43	13.2	109.3	107.7
173	M	56	24.54	0	2.01	5.36	0	0.43	4.5	0	1.58	0.86	0	0	0	29.4	158.0	154.4
174	M	53	20.84	0	1.29	2.65	0	0	1	0	0.86	0.86	0	0.43	0.79	25.4	236.6	461.7
177	F	52	23.52	0	1.43	3.5	0	0.43	2.5	0	1	1	0	0	0	14.4	138.0	113.9
178	F	53	26.56	0	0.43	3.93	0	0.43	2.5	0	0	1	0	0	0.43	50.1	129.5	259.5
179	M	57	26.58	0	0.86	1.43	0	0.43	0	0	0.43	1	0	0	0.43	26.4	60.2	140.3
182	F	54	28.83	0	1	2.5	0	0	0	0	1	2.5	0	0	0	12.5	42.5	108.8

The scoring system is described in Supplementary Table 3.

BMI, Body mass index; Yellow shading, volunteers chosen for the particular analysis

a, Total rye foods, sum of rye bread, rye pasta and rye flakes consumption

b, Rye flakes, porridge or muesli

c, Wash-out, 4-week wash-out period where volunteers avoided all wholegrain foods

d, Dose 1, volunteers consumed 3 servings of wholegrain rye foods per day (~ 48g wholegrain rye per day) for 4 weeks

e, Dose 2, volunteers doubled their intake of the same foods for a further 4 weeks (96 g wholegrain rye per day).

f, AR: Total Alkylresorcinol levels in plasma [12]

Supporting Information Table S3. The scoring system used to score the volunteers in terms of the frequency of consumption for each wholegrain rye product based on analysis of FFQ information

Frequency	Rating (FFQ Score)
Never (≤ 1 per week)	0
1 a week	0.14
2-4 per week	0.43
5-6 per week	0.78
Once a day	1
2-3 per day	2.5
4-5 per day	4.5
6+ per day	6

Supporting Information Table S4. Fragmentation analysis using Flow Infusion Electrospray-Ionization tandem Mass Spectrometry (FIE-MS_n) including use of pure chemical standards

Nominal mass	Sample MS/MS (<i>m/z</i>)	Standard	Standard MS/MS (<i>m/z</i>)	Match?
342a	Glucuronide ¹ ions (175 (MS ³ 113) and 113 (MS ³ 85)), glucuronide-aglycone ions (166 (MS ³ 148, 124, 122, 118*, 108))	2-Hydroxy-N-(2-hydroxyphenyl)acetamide (HHPAA) [M-H] ¹⁻	No standard available	-
342b	Sulfate ² ions (262*, 244), Sulfate-aglycone ions (262 (MS ³ 244, 218*, 200, 191, 135))	Unknown	Unknown	-
230	Sulfate ions (150 (MS ³ 108))	N-(2-hydroxyphenyl)acetamide (HPAA) [M-H] ¹⁻	150 (MS ² 108))	Match
433	Glucuronide ¹ ions (175 (MS ³ 113) and 113 (MS ³ 85)), glucuronide-aglycone ions 257 (MS ³ 239*, 211, 177, 151*, 129, 127, 97)	Unknown	Unknown	-
401a	Glucuronide ¹ ions (175 (MS ³ 113) and 113 (MS ³ 85)), glucuronide-aglycone ions (225 (MS ³ 207, 181, 167*, 153, 137, 80)	Unknown	Unknown	-
401b	Sulfate ² ions 321* Sulfate-aglycone ions (321 (MS ³ 303*, 217, 123)	Unknown	Unknown	-
340	Glucuronide ¹ ions (175 (MS ³ 113) and 113 (MS ³ 85)), glucuronide-aglycone ions 164 (MS ³ 136*, 118, 108)	2-hydroxy-2H-1,4-benzoxazin-3(4H)-one (HBOA) [M-H] ¹⁻	164 (MS ² 136*, 118, 108)	Match
399	381*, 355, 341, 319, 279, 263* (MS ³ 245, 145*, 127), 223, 193	Phenylacetylglutamine [M-H] ¹⁻	263 (MS ² 245, 145*, 127)	Match
148	130 (MS ³ 102, 86*, 85, 59), 86	Creatinine [M+Cl] ¹⁻	148 (MS ² 130 (MS ³ 102, 86*, 85), 86)	Match
330	Sulfate ² ions 250 (MS ³ 206*, 191, 177, 175, 149, 119, 100*)	Feruloylglycine (FG) [M-H] ¹⁻	250 (MS ² 206*, 191, 177, 175, 149, 100*)	Match
246	Sulfate ² ions 166 (MS ³ 148, 124*, 122, 118*, 108)	2-Hydroxy-N-(2-hydroxyphenyl)acetamide (HHPAA) [M-H] ¹⁻	No standard available	-

¹ Identified as a glucuronide with the aglycone [M-H-176]¹⁻ and an ion at *m/z* 175 ([M-2H- aglycone]¹⁻). MS³ fragment ions at *m/z* 113 (loss of CO₂ and H₂O from *m/z* 175), and anion at *m/z* 85 (loss of CO from *m/z* 113)

² Identified as a sulfonated molecule due to the presence of a ³⁴S isotope. Plus the fragment ion [M-H SO₃]¹⁻ (loss of SO₃, *m/z* 80).

* Most intense peak

a and b indicate there were two signals in the bin