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Cellular *in vitro* bioactivity of protein hydrolysates from brewers' spent grain

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Protein hydrolysates have been used as components of nutrition products, including geriatric and sports products, and in weight-control diets[(1)]. Brewers' spent grain (BSG), a co-product of the brewing industry, represents a unique source of protein hydrolysates. The aim of this study was to assess the *in vitro* bioactivity of BSG hydrolysates and fractionated hydrolysates.

Hydrolysates (designated U-W) were prepared from BSG using either Alcalase 2.4L, Corolase PP or Flavourzyme. Cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in both U937 and Jurkat T cells. The antioxidant activity of the hydrolysates was determined in U937 cells by two methods – ability to protect against hydrogen peroxide (H_2O_2)-induced (100 μM 60 min) oxidative stress, by the SOD assay and H_2O_2-induced (50 μM × 30 min) DNA damage, by the comet assay. An enzyme-linked immunosorbent assay (ELISA) was used to measure the effect of the samples on concanavalin-A (con-A) stimulated production of interferon-γ (IFN-γ) in Jurkat T cells, indicating their immunomodulatory potential.

	SOD activity (% of control)		DNA damage (% tail DNA)		IFN-γ production (% of control)	
	Mean	se	Mean	se	Mean	se
Control	100.0	0.0	5.1	0.6	100.0	0.0
H_2O_2 control	57.2*	0.7	41.8*	4.8	n/a	n/a
U	67.2	5.4	52.2	5.3	77.7†	2.3
U < 3 kDa	101.9#	4.9	42.3	2.3	98.1	2.8
U < 5 kDa	111.5#	3.3	41.3	1.8	102.6	3.8
U > 5 kDa	80.5#	4.3	43.7	3.1	82.0†	2.3
V	62.8	3.5	46.3	3.6	86.9†	1.4
V < 3 kDa	65.9	7.1	37.3	5.5	99.6	2.1
V < 5 kDa	67.2	4.1	33.2	4.0	105.6	3.6
V > 5 kDa	92.0#	2.8	32.7	5.1	81.3†	2.1
W	70.8	4.2	39.2	0.7	87.3†	1.5
W < 3 kDa	124.4#	2.9	29.1	3.1	113.5	4.1
W < 5 kDa	87.7#	3.5	24.0#	4.7	95.12	3.2
W > 5 kDa	108.4#	1.8	37.3	3.5	78.6†	1.6

Values are mean of three independent experiments. Statistical analysis by ANOVA followed by Dunnett's test. * Denotes significant difference in SOD activity/DNA damage, relative to control ($P<0.05$). # Denotes significant difference in SOD activity/DNA damage, relative to H_2O_2 control ($P<0.01$). † Denotes significant reduction in IFN-γ production, relative to control ($P<0.05$).

BSG protein hydrolysates were more cytotoxic in U937 than in Jurkat T cells (data not shown). Addition of H_2O_2 to U937 cells decreased SOD activity to 57.2% and increased % tail DNA to approximately 41.8% ($P<0.05$). Lowest molecular weight (m.w.) hydrolysates (<3,<5 kDa) showed strong protection against SOD reduction ($P<0.01$), particularly for fractionated hydrolysates of U and W. Only W<5 kDa significantly ($P<0.01$) repaired H_2O_2-induced DNA damage. Contrastingly, unfractionated hydrolysates and hydrolysates with higher m.w. (>5 kDa) possessed significant ($P<0.05$) anti-inflammatory potential, reducing IFN-γ production by up to 22.3%.

In conclusion, this study suggests BSG protein hydrolysates have bioactive potential; with low m.w. and higher m.w. fractionated hydrolysates demonstrating antioxidant and anti-inflammatory effects, respectively. These hydrolysates represent novel bioactive ingredients for inclusion in functional foods.

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1. McCarthy AL, OCallaghan YC & OBrien NM (2013) *Agriculture* **3**, 112–130.