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The immunomodulatory potential of *in vitro* digested low-fat milk supplemented with brewers' spent grain protein hydrolysate; selection of a non-cytotoxic level of digestate

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Protein hydrolysates from agricultural crops have shown encouraging bioactive and techno-functional characteristics that may be used in the development of functional foods[(1)]. It is important that bioactive protein hydrolysates demonstrate an ability to retain their bioactivity during digestion. Brewers' spent grain (BSG), a by-product of the brewing industry, is a potential source for the development of protein hydrolysates. The aim of this study was to incorporate a bioactive, BSG-derived protein hydrolysate into commercially available low-fat milk and assess the cytotoxicity and immunomodulatory effects of digestates, following *in vitro* gastrointestinal digestion.

Hydrolysate U was obtained on Alcalase 2·4L digestion of a BSG protein-rich isolate. The hydrolysate was freeze-dried whole (U) or fractionated using 5 and 3 kDa molecular cut-off membranes, and permeates and retentate were designated U, U < 3, U < 5 and U > 5. Samples were added to low-fat milk at a concentration of 0·125% (v/v). A static gastrointestinal digestion model, as previously described[(2)] was used to mimic human digestion. The (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay was used to assess the effect of digestates (0–10% ; v/v) on Jurkat T cell proliferation. The effect of digestates on interferon-gamma (IFN-gamma) and interleukin-6 (IL-6) secretion in concanavalin-A (con-A) stimulated Jurkat T cells was measured by ELISA. Data were expressed as a percentage of untreated (control) cells.

	Control		1% digestate		2% digestate		2·5% digestate		5% digestate		10% digestate	
	Cell Viability (%)											
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Digestate blank	95·3	5·8	108·2	2·8	121·3	7·2	120·6	14·8	117·9	6·2	126·7	3·5
Unfortified milk	96·9	2·8	104·1	2·6	106·6	3·2	98·3	12·8	77·0	18·5	43·6	35·2
U	92·7	7·4	102·1	7·0	110·8	4·0	104·2	3·7	77·8	19·0	23·6*	7·7
U < 3	101·4	1·7	109·3	9·2	110·2	18·0	98·1	1·8	81·1	14·7	29·1*	21·0
U < 5	112·8	6·2	100·8	4·9	103·0	7·0	98·9	10·7	73·8	17·5	33·0*	27·2
U > 5	101·0	1·8	99·6	6·6	99·4	5·0	94·0	3·5	29·7*	13·8	4·2*	1·4

Values are mean of three independent experiments. Statistical analysis by ANOVA followed by Dunnett's test.
* Denote significant difference in cell viability, relative to untreated Jurkat cells ($P < 0·05$).

Treatment with digestates of milk with added hydrolysates U, U < 3 and U < 5 at 10% (v/v) and hydrolysates U > 5 at 5% and 10% (v/v), for 24 hours significantly reduced viability of Jurkat T cells. Following on from the cytotoxicity results, the highest non-toxic concentration of digestates (2·5%) was selected for further investigation. Preliminary results suggest that milk digestates with added >5 kDa hydrolysate can decrease IFN-gamma and IL-6 secretion in stimulated Jurkat T cells (data not shown). In conclusion, these results suggest that low-fat milk fortified with BSG hydrolysate can attenuate cytokine production in stimulated Jurkat T cells.

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