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Potential bioactivity of hydrolysates derived from bovine lung

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Increasing life expectancy, coupled with rising health care costs, necessitates the development of foods with potential health benefits; hence, novel bioactives for functional food development are highly sought. The meat industry produces a large quantity of protein rich co-products that are often discarded or sold as low value animal feed. Bovine lung tissue contains large amounts of proteins including collagen which, when hydrolysed is rich in peptides with bioactivities[(1)]. Bioactive peptides are small protein fragments that can impart physiological benefits to the consumer after ingestion. The objective of this study was to determine the effect of bovine lung hydrolysates in human U937 monocytes and RAW264·7 mouse macrophages.

Three hydrolysates were produced from bovine lung using the food grade enzymes papain (A), pepsin (B), and alcalase (C). Cell lines were treated with increasing concentrations of the hydrolysates for 24 h and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell viability and to calculate the concentration of hydrolysate that inhibited viability by 50 % (IC_{50} values). In addition, oxidative DNA damage was evaluated in U937 cells using the comet assay, following pre-treatment of the cells with 0·1 % (w/v) of the hydrolysates for 24 h and subsequent exposure of the cells to 80 μM H_2O_2 for 30 min. The immunomodulatory potential of the hydrolysates was determined based on their ability to reduce the production of the pro-inflammatory cytokines interleukin 6 (IL-6) and interleukin 1β (IL-1β) in lipopolysaccharide (LPS) stimulated RAW264·7 cells. RAW264·7 cells were exposed to hydrolysates at 0·05 and 0·005 % (w/v) in the presence of LPS for 24 hours. Cytokine production was then measured using ELISA and results were expressed as a percentage of LPS stimulated cells.

Table 1. Cytokine production (% of control)

	Interleukin 6				Interleukin 1β			
	0·05 %		0·005 %		0·05 %		0·005 %	
Samples	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	100	0·0	100	0·0	100	0·0	100	0·0
A	77·2*	1·7	74·8*	6·1	47·1*	2·8	71·7*	0·2
B	102·1	6·1	95·6	2·4	93·5	4·4	82·0	5·9
C	42·4*	3·1	58·7*	2·0	35·9*	0·6	74·6*	4·9

Table 2. DNA damage (% Tail DNA)

Samples	Mean	SE
Control	11·9	1·4
H_2O_2	70·6	2·8
A	60·9	3·3
B	60·1	6·9
C	64·0	0·5

Values are mean of two or three independent experiments. Statistical analysis was by ANOVA followed by Dunnett's test. * $P < 0·05$

Hydrolysate B did not demonstrate immunomodulatory potential. However, hydrolysates A and C when assayed at concentrations of 0·05 and 0·005 % (w/v) were found to significantly ($P < 0·05$) decrease IL-6 and IL-1β production in RAW264·7 cells (Table 1). The hydrolysates did not demonstrate antioxidant activity in the comet assay and no significant protection against the H_2O_2 challenge was observed (Table 2). In conclusion, results indicated that papain- or alcalase-hydrolysed bovine lung tissue may have potential anti-inflammatory activity.

1. Lafarga T, Hayes M. (2014). Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Science* **98** (2), 227–239.