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In vitro antioxidant and anti-inflammatory effects of brewers' spent grain protein rich isolate and its associated hydrolysates

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ABSTRACT

Brewers' spent grain (BSG) is a protein-rich by-product of the brewing industry. The present study examined the *in vitro* bioactivity of a BSG protein enriched preparation and its associated enzymatic hydrolysates (assigned A–J). Cytotoxicity was measured using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay in U937 and Jurkat T cells. IC_{50} values were lower in the U937 cell line, ranging from 4.93 to 9.27% v/v versus a range of 4.11% v/v to undetectable in Jurkat T cells. The superoxide dismutase (SOD) and comet assays were performed on U937 cells pre-incubated with test samples and subsequently exposed to an oxidant. Hydrogen peroxide (H_2O_2) significantly reduced SOD activity by 37.7% and none of the test samples provided protection. None of the samples protected against DNA damage induced by *tert*-butylhydroperoxide (*t*-BOOH); hydrolysate H, prepared with Alcalase at 60 °C, protected against H_2O_2-induced DNA damage. The total phenolic content (TPC) was found to range from 0.021 to 0.055 mg GAE/mg dry powder. The effect of the BSG-derived test samples on cytokine production (IL-2, IL-4, IL-10, IFN-γ) in Concanavalin A (conA) stimulated Jurkat T cells was measured using an enzyme linked immunosorbent assay (ELISA). The samples had no effect on IL-2 and IL-4 production. The unhydrolysed sample C significantly reduced IL-10, while the protein rich isolate, unhydrolysed control samples and hydrolysates D, E, F, and J significantly reduced IFN-γ production. The BSG preparations possess little antioxidant potential and exhibit selective immunomodulatory effects that may be of benefit in the control of inflammatory diseases.

1. Introduction

Brewers' spent grain (BSG) is the residual solid fraction of barley malt remaining after wort is produced in the brewing process. Although BSG is mainly used as an animal feed, options for further uses are being explored. In recent years, there has been an unprecedented and extensive demand for inexpensive plant-derived proteins and bioactive ingredients for human consumption. The protein content of BSG is approximately 20% (w/dw) (Mussatto, Dragone, & Roberto, 2006), and this has potential to be exploited for human nutrition.

Protein hydrolysates have long been the focus of nutritional research for their ability to act as a source of biofunctional agents. Different protein hydrolysates have been shown to exert antioxidant and anti-inflammatory effects; these include pea protein (Ndiaye, Vuong, Duarte, Aluko, & Matar, 2012), soy protein (Kong, Guo, Hua, Cao, & Zhang, 2008; Peñta-Ramos & Xiong, 2002), fish protein (Harnedy & FitzGerald, 2012) and casein hydrolysates (Lahart, O'Callaghan, Aherne, O'Sullivan, FitzGerald, & O'Brien, 2011; Phelan, Aherne-Bruce, O'Sullivan, FitzGerald, & O'Brien, 2009). Protein hydrolysates have numerous uses in human nutrition ranging from protein supplementation of geriatric and sports nutrition products, energy drinks and weight-loss diets to clinical applications including treatment of Crohn's disease, liver disease and ulcerative colitis (Clemente, 2000). While much evidence exists with regard to protein hydrolysates from the aforementioned sources, to our knowledge, this is the first study on the bioactivity of protein preparations from BSG.

The antioxidant activity of a compound can be measured using an array of methods, both cellular and non-cellular. Superoxide dismutases are a group of metalloenzymes that catalyse the conversion of the superoxide anion ($O_2^{\bullet -}$) to molecular oxygen and hydrogen peroxide (H_2O_2), thus forming an essential part of the cellular antioxidant defence system. The single-cell gel electrophoresis assay (comet assay) is also used to determine antioxidant potential, through the induction of DNA damage (single-strand break) by an oxidant. In these assays,

Abbreviations: ANOVA, analysis of variance; BSG, brewers' spent grain; conA, concanavalin A; EtBr, ethidium bromide; FBS, foetal bovine serum; FDA, fluorescein diacetate; GAE, gallic acid equivalents; H_2O_2, hydrogen peroxide; IC_{50}, half maximal inhibitory concentration; LMP, low melting point; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide; $O_2^{\bullet -}$, superoxide anion; SOD, superoxide dismutase; *t*-BOOH, *tert*-butylhydroperoxide; Th1, T helper 1; Th2, T helper 2; TPC, total phenolic content.

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the antioxidant activity is determined by the ability of a compound to protect against oxidant-induced damage. Common oxidants employed in cellular antioxidant assays include *tert*-butylhydroperoxide (*t*-BOOH) and H_2O_2 (Aherne & O'Brien, 2000a, 2000b; Alia, Ramos, Mateos, Bravo, & Goya, 2005; Park, Yang, Yoon, & Lee, 2003).

Cytokines are low molecular weight cell-signalling protein molecules including interleukins, interferons and tumour necrosis factor, which play a role in the inflammatory response. The ability of a compound to alter cytokine production may indicate anti-inflammatory potential and thus it may have beneficial effects for example, in inflammatory diseases such as atherosclerosis and rheumatoid arthritis. Jurkat T cells have been extensively used as a model for immunomodulatory studies (Benbernou, Esnault, Shin, Fekkar, & Guenounou, 1997; Grassberger et al., 1999; Phelan et al., 2009).

The aims of the present study were: firstly, to assess the potential bioactivity of a protein rich isolate from BSG and its associated hydrolysates by measuring their cytotoxic and antioxidant effects in human monocytic blood cells (U937); secondly, to measure the ability of the BSG protein rich isolate/hydrolysates to protect against oxidant-induced DNA damage in the U937 cell line; and thirdly, to determine potential immunomodulatory effects of the BSG protein isolate/hydrolysates in concanavalin A (con-A) stimulated human Jurkat T cells.

2. Materials and methods

2.1. Materials

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich Ireland Limited, Co. Wicklow, Ireland. U937 and Jurkat T cells were obtained from the European Collection of Animal Cell Cultures (ECACC).

2.2. Generation of protein rich isolate and protein hydrolysates

For production of the protein rich isolate (49.13% protein dw), 940 g BSG was first sheared for 20 s at 11,000 rpm using an Ultra Turrax® T25 basic high performance disperser (IKA® Werke GmbH & Co. KG, Staufen, Germany) in 5.0 L dH_2O. The sheared samples were then allowed to stir gently for 1 h at room temperature prior to centrifugation at 2700 *g* for 20 min at 10 °C (Hettich Zentrifugen Universal 320R centrifuge, Andreas Hettich GmbH & Co., Tuttlingen, Germany). The precipitate obtained from the above process was subjected to two sequential 1 h extractions in 5.0 L 110 mM NaOH at 50 °C. The combined supernatants from the above extractions obtained after centrifugation were adjusted to pH 3.8, stirred gently for 15 min at room temperature and then centrifuged. The precipitated protein obtained was re-suspended in dH_2O, neutralised to pH 7.0 using 2 N NaOH and freeze-dried.

The freeze dried protein rich isolate (49.13% protein dw) was hydrolysed with the commercially available enzymes Corolase PP, Flavourzyme and Alcalase 2.4 L under conditions described in Table 1. The pH was maintained by addition of 0.5 N NaOH using a pH-Stat system (Metrohm 718 STAT Titrino, Herisau, Switzerland) and the temperature was kept constant using a thermostatically controlled water bath (IKA® Werke GmbH & Co. KG, Staufen, Germany). The resulting hydrolysates were each adjusted to pH 7.0, heated at 95 °C for 10 min to inactivate the enzyme and were then freeze dried. Prior to analysis, freeze-dried samples were made up to 1% (w/v) solution with distilled deionised water, filter sterilised, aliquoted into sterile eppendorfs and stored at −20 °C.

Table 1
Preparation of protein isolate/hydrolysate test samples from brewers' spent grain (BSG).

Sample	Description	Enzyme/water	E/S ratio	Temperature (°C)	pH	Time (h)
A	BSG protein rich isolate	–	–	–	–	–
B	Control for D, E, F, G, J	Water	–	50	7	4
C	Control for H, I	Water	–	60	9	4
D	BSG protein hydrolysate	Corolase PP	1% (w/w)	50	7	4
E	BSG protein hydrolysate	Corolase PP	2.5% (w/w)	50	7	4
F	BSG protein hydrolysate	Flavourzyme	1% (v/w)	50	7	4
G	BSG protein hydrolysate	Flavourzyme	2.5% (v/w)	50	7	4
H	BSG protein hydrolysate	Alcalase 2.4 L	1% (v/w)	60	9	4
I	BSG protein hydrolysate	Alcalase 2.4 L	2.5% (v/w)	60	9	4
J	BSG protein hydrolysate	Alcalase 2.4 L	2.5% (v/w)	50	7	4

2.3. Total phenolic content (TPC)

The total phenolic content (TPC) of the protein rich isolate and its associated hydrolysates was measured using the Folin–Ciocalteu method as described previously (Singleton & Rossi, 1965). This assay measures the ability of a compound to reduce the yellow oxidising Folin–Ciocalteu reagent to a blue/green colour. Absorbance is measured spectrophotometrically at 765 nm and results are expressed as mg gallic acid equivalents per mg dry powder (mg GAE/mg dry powder).

2.4. Cell culture

U937 cells, a human monocytic blood cell line, and Jurkat T cells, a human leukaemic T cell line, were maintained in antibiotic-free RPMI-1640 medium supplemented with 10% (v/v) foetal bovine serum (FBS), at 37 °C in a 5% CO_2 atmosphere. Cell densities of 1×10^5 cells/mL (cytotoxicity and superoxide dismutase assays) or 2×10^5 cells/mL (comet and enzyme-linked immunosorbent assays) were used for experimentation. Reduced serum media (2.5% FBS) was used for all experiments.

2.5. Cell proliferation

To measure cell proliferation the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out in both the U937 and Jurkat T cell lines. Cells (1×10^5 cells/mL) were incubated for 24 h at 37 °C with test samples (A–J) at concentrations ranging from 0 to 20% v/v. To account for the colour of the samples, a media blank was also included (samples and media only, no cells). The MTT assay kit was then used to quantify cell proliferation. In this assay, MTT (a yellow tetrazolium salt) is converted to a formazan derivative (purple colour), and the absorbance of the converted dye is measured at 570 nm. Briefly, 10 μL MTT reagent 1 and 100 μL RPMI were added to cells and incubated for 4 h at 37 °C. Following incubation, 100 μL was removed from the wells and 100 μL MTT reagent 2 was added. A further incubation period of 24 h at 37 °C followed. Absorbance was read at 570 nm using a microplate reader (Spectrafluorplus, Tecan). Using cell proliferation data from the MTT assay, IC_{50} values (the concentration of a test sample that induces 50% cell death) were determined. GraphPad Prism 4 was used for analysis and IC_{50} values are expressed as % (v/v). On the basis of the MTT assay and published literature (Cumby, Zhong, Naczk, & Shahidi, 2008; Lahart et al., 2011; Piccolomini, Iskandar, Lands, & Kubow, 2012), a non-toxic concentration of 0.5% (v/v) BSG protein preparations was used for further analyses.