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In vitro cellular bioactivities of Maillard reaction products from sugar-gelatin hydrolysate of unicorn leatherjacket skin system

Supatra Karnjanapratum [a], *Yvonne C. O'Callaghan* [b], *Soottawat Benjakul* [a], *Nora M. O'Brien* [b,*]

[a] *Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University. Hat Yai, Songkhla 90112, Thailand*

[b] *School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

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ABSTRACT

The effects of Maillard reaction products (MRP), produced from galactose and a gelatin hydrolysate which was derived from unicorn leatherjacket skin, on *in vitro* cellular antioxidant activity, immunomodulatory properties and anti-cancer activity were investigated. MRP (750–1500 μg/mL) protected against H_2O_2-induced DNA damage in U937 cells. DNA damage was reduced by approximately 50% at the highest MRP concentration (1500 μg/mL). The activities of cellular antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were decreased in HepG2 cells exposed to H_2O_2 but were induced in the presence of MRP. MRP reduced the production of pro-inflammatory cytokines (IL-1β and IL-6) and nitric oxide in LPS-induced RAW264.7 cells. The proliferation of colon carcinoma, Caco-2 cells was inhibited in the presence of MRP with an IC_{50} of 1.7 mg/mL. Therefore, MRP demonstrated bioactive potential through the suppression of oxidative stress and inflammation *in vitro* in addition to inhibiting the proliferation of human colon cancer cells.

1. Introduction

Maillard reaction products (MRPs) have been proven to be potential candidates as effective antioxidants (Amarowicz, 2009). Maillard derived antioxidants can be prepared using various model systems, such as chitosan-sugar, porcine plasma protein–glucose and whey protein-reducing sugars (Lertittikul, Benjakul, & Tanaka, 2007; Liu, Li, Kong, Li, & Xia, 2014; Phisut & Jiraporn, 2013). Glycosylation of fish protein hydrolysates with monosaccharides or oligosaccharides has been performed under various conditions. The antioxidant activities of a protein hydrolysate from silver carp were effectively improved using Maillard reaction under the powdered state (You, Luo, Shen, & Song, 2011). In addition to their antioxidant potential, MRPs were also demonstrated to exhibit promising immunomodulatory and anti-tumour effects. Teodorowicz, Fiedorowicz, Kostyra, Wichers, and Kostyra (2013) reported that the Maillard reaction between glucose and peanut 7S globulin resulted in a product which reduced pro-inflammation in intestinal cells (Caco-2 cells) as evidenced by a reduction in IL-8 secretion. Song, Wei, Zhang, Yang, and Wang (2011) reported that the anti-proliferative activity of pepsin hydrolysate from half-fin anchovy was increased following heat treatment, in the absence of added sugar, in DU-145 human prostate cancer cells, 1299 human lung cancer cells and 109 human

* *Corresponding author.* School of Food and Nutritional Sciences, University College Cork, Cork, Ireland. Tel.: +353 21 4902884; fax: +353 21 4270244.
E-mail address: nob@ucc.ie (N.M. O'Brien).
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oesophagus cancer cells. Maillard reaction products have also caused the production of some undesirable compounds including cytotoxic substances. The cytotoxic effects of MRP have been reported in different model systems and are associated with the use of high temperatures in the production of the MRP (Jing & Kitts, 2002).

Recently, gelatin hydrolysate from the skin of unicorn leatherjacket with antioxidant activity has been successfully produced using an autolysis assisted process in combination with glycyl endopeptidase (Karnjanapratum & Benjakul, 2015). The resulting gelatin hydrolysate also possessed antioxidant properties in *in vitro* cellular model system (Karnjanapratum, O'Callaghan, Benjakul, & O'Brien, 2015b). To enhance the antioxidant activity as well as other bioactivities, the use of Maillard reaction to prepare Maillard reaction products (MRPs) based on gelatin hydrolysate may be a promising and effective technique. Antioxidant MRPs from gelatin hydrolysate (GH) of unicorn leatherjacket skin were prepared via glycation with different types of saccharides under optimal conditions (Karnjanapratum, O'Brien, & Benjakul, 2015a). Among all MRPs tested, the MRP produced from a mixture of GH and galactose (2:1, w/w) by heating at 70 °C and 55% relative humidity for 36 h showed the highest antioxidant activity (Karnjanapratum et al., 2015a). To date, there have been no reports on the cellular bioactivities of MRPs derived from fish gelatin hydrolysate in combination with a sugar system. Therefore, the present study aimed to investigate the antioxidant activity, immunomodulatory potential and anti-cancer effects of a Maillard reaction product (MRP) derived from gelatin hydrolysates of unicorn leatherjacket skin in cell culture model systems in order to assess the potential of this MRP as a functional food ingredient. The antioxidant potential of the MRP following *in vitro* gastrointestinal digestion was also investigated to determine its stability during gastrointestinal digestion.

2. Materials and methods

2.1. Chemical

Cell growth media and other solutions were purchased from Sigma-Aldrich Chemical Co. (Dublin, Ireland). Mouse leukaemic macrophages (RAW264.7 cells), human histiocytic lymphoma cells (U937 cells), human hepatoma cells (HepG2 cells) and human colon cancer cells (Caco-2 cells) were purchased from the European Collection of Animal Cell Cultures (Salisbury, UK). Foetal bovine serum (FBS) was purchased from Invitrogen (Paisley, Scotland). Costar cell culture plastics were supplied by Fisher Scientific (Dublin, Ireland). 2,2′-Azinobis (3-thylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid (Trolox), cell culture chemicals and reagents were from Sigma Chemical Co. (Dublin, Ireland).

2.2. Preparation of Maillard reaction product (MRP) from gelatin hydrolysate

2.2.1. Preparation of gelatin hydrolysate from unicorn leatherjacket skin

Gelatin hydrolysate was prepared from unicorn leatherjacket skin using an autolysis-assisted process and partial purified glycyl endopeptidase (8%, based on solid matter) as described by Karnjanapratum and Benjakul (2015). The resulting gelatin hydrolysate was referred to as 'GH'.

2.2.2. Preparation of Maillard reaction product (MRP)

MRP was prepared as described by Karnjanapratum et al., (2015a). Briefly, the powder of GH was mixed with galactose (2:1, w/w) and the mixture was heated at 70 °C with 55% relative humidity for 36 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting MRP was collected and subjected to analyses.

2.3. Cell culture

U937 cells were grown in RPMI-1640 medium supplemented with 100 mL/L FBS. RAW264.7 and HepG2 cells were maintained in DMEM supplemented with 100 mL/L FBS. Caco-2 cells were cultured in DMEM supplemented with 100 mL/L FBS and 10 mL/L non-essential amino acids. Cells were cultured following the method described by Karnjanapratum et al. (2015b).

2.3.1. Cell proliferation

U937, RAW264.7 and HepG2 cells (5×10^4 cells/mL) was supplemented with increasing concentrations of MRP in 96-well flat-bottom plates with a final volume of 200 μL at 37 °C for 24 h. Cell proliferation was determined by means of the MTT assay as described in Karnjanapratum et al. (2015b).

2.4. Determination of antioxidant activity in cell model systems

2.4.1. Determination of DNA damage (Comet assay)

U937 cells (1×10^5 cells/mL) were treated with MRP (750, 1000 and 1500 μg/mL) for 24 h in a 24-well plate with a final volume of 1 mL media, containing reduced FBS (25 mL/L) at 37 °C. Following incubation, cells were treated with 40 or 60 μM H_2O_2 for 30 min. Oxidative DNA damage in the U937 cells was assessed using the Comet assay as described by McCarthy et al. (2012).

2.4.2. Antioxidant enzyme activity assays: superoxide dismutase (SOD) and catalase (CAT) activities

HepG2 cells (2×10^5 cells/mL, 5 mL) were incubated with different concentrations of MRP (250 and 500 μg/mL) for 24 h at 37 °C. Following incubation, cells were exposed to 2 mM H_2O_2 for 2 h. Cells were harvested, sonicated and centrifuged (10,000 × g, 30 min) at 4 °C, and the supernatant was collected for the determination of antioxidant enzyme activity. The activity of total cellular SOD and CAT was determined using the method described in Karnjanapratum et al. (2015b).

2.5. Immunomodulatory effect of Maillard reaction product

2.5.1. Cytokine production: interleukin-6 (IL-6) and interleukin-1β (IL-1β)

RAW264.7 cells, at a density of 2×10^5 cells/mL, were seeded in 96-well plates in the presence of lipopolysaccharide (LPS, 0.02 μg/mL for IL-6 and 0.1 μg/mL for IL-1β) and treated with