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In vitro α-glucosidase, angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory properties of brewers' spent grain protein hydrolysates

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ABSTRACT

The *in vitro* α-glucosidase, α-amylase, dipeptidyl peptidase IV (DPP-IV) and angiotensin converting enzyme (ACE) inhibitory activities of pale brewers' spent grain protein enriched isolate (BSG-PI) hydrolysates were studied. Eleven commercially available enzyme preparations derived from papaya, porcine pancreas, *Aspergillus oryzae* and *Bacillus licheniformis* were used to generate the hydrolysates. The hydrolysates had degree hydrolysis (DH) values ranging from 1.15 to 14.67% and the DH values obtained correlated well with hydrolysate molecular mass distribution profiles. Reverse-phase HPLC analysis of the BSG-PI and its hydrolysates demonstrated considerable variation in peptide composition. While tryptic digests of BSG-PI resulted in the greatest α-glucosidase inhibitory activity, no significant change in α-amylase inhibition was observed with the hydrolysates. Digestion with Corolase PP resulted in the highest DPP-IV inhibition (75 ± 3.06% at 3.5 mg mL^{-1}) while the Prolyve 1000 hydrolysate displayed highest ACE inhibition (89.25 ± 2.46% at 1 mg mL^{-1}). The results obtained highlight the potential role of the BSG-PI hydrolysates as functional food ingredients in the management of type II diabetes and hypertension.

1. Introduction

The prevalence of type II diabetes has increased dramatically in the last number of years. By 2030 an estimated 366 million people will be affected worldwide with approximately 70% of subjects also suffering from hypertension (Lago, Singh, & Nesto, 2007; World Health Organization, 2006). Hyperglycaemia, a common symptom of type II diabetes, is a condition characterized by a rapid increase in post-prandial serum glucose. This may arise due to hydrolysis of starch by pancreatic α-amylase which is further hydrolysed by intestinal α-glucosidase to release glucose (Adisakwattana, Lerdsuwankij, Poputtachai, Minipun, & Suparpprom, 2011; Jo et al., 2011). Postprandial hyperglycaemia can be delayed by inhibiting these enzymes resulting in improved glycaemic control in diabetic patients (Adisakwattana et al., 2011).

Another strategy developed for the management of type II diabetes involves the incretin hormones, glucose dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). The incretins can enhance insulin secretion from pancreatic beta cells in the presence of glucose *in vivo* (Drucker, 2006). However, these hormones are quickly degraded by dipeptidyl peptidase IV (DPP-IV) resulting in a decreased insulin response (Nongonierma & FitzGerald, 2012). *In vivo* studies have shown an increase in circulating GLP-1 by a factor of 4 to 6 following the intake of DPP-IV drug inhibitors (Nauck & El-Ouaghlidi, 2005). Numerous food peptide-derived inhibitors of DPP-IV having Xaa-Pro, Pro-Xaa or Ala-Xaa (with Xaa an amino acid residue) have been reported in the literature indicating that Pro and Ala residues are significant contributors to DPP-IV inhibition (Yan, Ho, & Hou, 1992). A study by Hejgaard, Jacobsen, and Svendsen (1991) using porcine pancreatic α-amylase, found that barley proteins did not inhibit α-amylase. However, to date no studies appear in the literature reporting on the inhibition of α-glucosidase and/or DPP-IV by barley protein hydrolysates.

Angiotensin converting enzyme (ACE) is a key activity which is targeted for inhibition during the treatment of hypertension (Norris, Casey, FitzGerald, Shields, & Mooney, 2012). ACE is a carboxypeptidase involved in the renin–angiotensin system (RAS) and kinin nitric oxide system (KNOS). In the RAS, ACE cleaves angiotensin I into angiotensin II, a potent vasoconstrictor while in the KNOS, ACE inactivates the hypotensive peptide bradykinin (Eriksson, Danilczyk, & Penninger, 2002). Therefore, excessive action of ACE leads to increased vasoconstriction and hypertension. The most effective food protein-derived ACE-inhibitory peptides are reported to contain Tyr, Phe, Trp and/or Pro at the C-terminal (Gomez-Ruiz, Ramos, & Recio, 2004). Barley has been reported to possess many of the currently known ACE inhibitory peptide sequences within its primary protein structures (Loponen, 2004). Given the predisposition of subjects with type II diabetes to develop hypertension, an effective strategy in the management of diabetes could be the development of multi-functional peptide inhibitors with the ability to inhibit a number of key enzymes, i.e., α-glucosidase, α-amylase,

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DPP-IV and ACE. Studies on preparing ACE inhibitory peptides from barley proteins are limited. However, using an *in silico* approach Gu, Majumder, and Wu (2011) identified a number of sequences within barley hordein with predicted IC_{50} values of <10 μM.

Pale BSG is a major by-product of the brewing industry and is composed of between 15 and 26% protein which is similar to that in the original barley grain. Hordeins account for 60% of the total protein in BSG and are recognised for their high glutamine and proline contents (Baxter, 1981). Glutelins, the barley cell wall structural proteins, comprise the bulk of the remaining protein fraction and contain high levels of Glu, Ala and Asp residues (Shewry & Miflin, 1983). Given the prevalence of Pro and Ala residues within the amino acid sequences of BSG proteins, it is likely that peptides with high ACE and DPP-IV inhibitory activity could be released during their hydrolysis. Pale BSG has recently become the focus for the development of functional and bioactive food ingredients (McCarthy et al., 2013; McCarthy et al., 2012). Being the major by-product of the brewing industry, pale BSG is a low-cost substrate widely available in large quantities.

Enzymatic hydrolysis of food proteins has been extensively utilised to generate peptides with bioactive potential (Harnedy & FitzGerald, 2013; Nongonierma & FitzGerald, 2013; Norris et al., 2012; Pihlanto, Virtanen, & Korhonen, 2010). An important step in this procedure is identifying proteolytic enzymes with appropriate specificity to release these bioactive peptides. With this in mind, the aim of this study was to evaluate the potential release of bioactive peptides from a BSG-PI using a range of commercially available proteolytic enzyme preparations. The BSG-PI hydrolysates were characterized with respect to their physicochemical properties and were also analysed *in vitro* for their ability to inhibit α-glucosidase, α-amylase, DPP-IV and ACE.

2. Materials and methods

2.1. Materials

The BSG samples obtained from a single batch which was brewed in November 2009, were collected from the brewery, vacuum-packed and stored in polypropylene bags at −20 °C until use. Alcalase 2.4 L (Alc) and Flavourzyme® 500 L (Flav) were obtained from Sigma Chemical Co., (Dorset, UK), Prolyve 1000 (Pro1k) was kindly supplied by Lyven Enzymes Industrielles (Caen, France), Protex 6 L (Pro6L) was obtained from the Genencor Division of Danisco (Rochester, NY, USA), Protamex™ (ProX) was kindly provided by Novo Nordisk (Bagsvaerd, Denmark), Corolase® PP (CorPP) and Corolase® L10 (CorL10) were obtained from AB Enzymes (Darmstadt, Germany). Promod™ 144MG (ProMG), Promod™ 439 (Pro439), Promod™ 24P (Pro24P) and Trypsin 250 (Tryp) were obtained from Biocatalysts Ltd. (Cefn Coed, Wales). Abz-Gly-p-nitro-Phe-Pro-OH, Abz-Gly-OH-HCl, H-Gly-Pro-7 amino-4-methyl coumarin (AMC) and Diprotin A were from Bachem Feinchemikalien (Bubendorf, Switzerland). HPLC grade water and acetonitrile were from VWR International (Dublin, Ireland) and trinitrobenzensulphonic acid (TNBS) was from Fischer Scientific (Dublin, Ireland). Porcine pancreatic α-amylase (EC 3.2.1.1; ≥10 units mg^{-1} solid), rat intestinal acetone powders of α-glucosidase (EC 3.2.1.20) and human DPP-IV (E.C. 3.4.14.5; ≥10 units mg^{-1} protein) were purchased from Sigma–Aldrich Co. Bovine lung for the purification of ACE was kindly provided by a local abattoir. All other chemicals were obtained from Sigma and were of analytical grade unless otherwise stated.

2.2. Generation of pale BSG protein-enriched isolate (BSG-PI) hydrolysates

The pale BSG-PI was obtained using an alkaline extraction procedure as previously described (Connolly, Piggott, & FitzGerald, 2013). The BSG-PI hydrolysates were prepared using the enzymes and reaction conditions as outlined in Table 1. In this table, 2.5Alc960 denotes hydrolysis carried out using 2.5% (v/w) Alcalase at pH 9.0 and 60 °C while 1.0Alc760 denotes hydrolysis carried out using 2.5% (v/w) Alcalase at pH 7.0 and 50 °C. Solutions (50 mL) of 3% ((w/v) on a protein basis) BSG-PI (50% (w/w) protein) were prepared and allowed to hydrate for 1 h at 20 °C after which the temperature was increased to 50 °C. The pH of the solution was then adjusted to the required pH, the sample was allowed to equilibrate for a further 30 min and the pH was readjusted if necessary. The proteolytic preparations at an enzyme:substrate ratio of (1 or 2.5% (w/w or v/w)) were added to the BSG-PI solutions which were vigorously stirred. After addition of the proteolytic preparation, the pH of the BSG-PI solution was kept constant at the required pH using a pH stat (718 stat Titrino, Metrohm, Herisau, Switzerland) by the addition of 0.5 N NaOH for a duration of 240 min. The enzymes were inactivated by heating the hydrolysate sample at 90 °C for 10 min in a water bath. Samples were then lyophilised using a Labconco Freeze Dry System/Freezone 4.5 (Labconco Corporation, Kansas City, MO, USA). Samples were stored at −20 °C prior to further analysis.

2.3. Quantification of degree hydrolysis (DH)

The DH, expressed as the percentage of peptide bonds hydrolysed, was determined in triplicate using the TNBS method (Adler-Nissen, 1979) as described by Spellman, McEvoy, O'Cuinn, and FitzGerald (2003) with minor modifications. Samples and standard solutions were prepared in 1% (w/v) SDS. Triplicate aliquots (0.125 mL) of test or standard solutions were added to test tubes containing 1.0 mL

Table 1
Test sample code, enzymatic preparation, enzyme source, enzyme:substrate (E:S), incubation temperature and pH used during the hydrolysis of brewers' spent grain protein-enriched isolate (BSG-PI).

Sample	Description	Enzyme source	E:S ratio (%)	Temp (°C)	pH
BSG-PI	Intact BSG Protein	–	–	–	–
2.5Alc960	Alcalase 2.4 L	*Bacillus licheniformis*	2.5 (v/w)	60	9
2.5Alc950	Alcalase 2.4 L	*Bacillus licheniformis*	2.5 (v/w)	50	9
1.0Alc760	Alcalase 2.4 L	*Bacillus licheniformis*	1.0 (v/w)	60	7
CorL10	Corolase L10	Papain	1.0 (v/w)	50	7
CorPP2.5	CorolasePP	Porcine pancreatic preparation	2.5 (w/w)	50	7
CorPP1.0	CorolasePP	Porcine pancreatic preparation	1.0 (w/w)	50	7
Tryp	Trypsin 250	Pancreatic enzyme	1.0 (w/w)	50	7
Flav2.5	Flavourzyme 500 L	*Aspergillus oryzae*	2.5 (v/w)	50	7
Flav1.0	Flavourzyme 500 L	*Aspergillus oryzae*	1.0 (v/w)	50	7
ProMG	Promod 144MG	Papain	1.0 (w/w)	50	7
Pro6L	Protex6L	*Bacillus licheniformis*	1.0 (w/w)	50	7
ProX	Protamex	*Bacillus licheniformis*	1.0 (w/w)	50	7
Pro24P	Promod 24P	*Bacillus licheniformis*	1.0 (w/w)	50	7
Pro439	Promod 439	*Bacillus licheniformis*	2.0 (w/w)	50	7
Pro1k	Prolyve 1000	*Bacillus licheniformis*	1.0 (w/w)	50	7