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Quinoa (*Chenopodium quinoa* Willd.) protein hydrolysates with *in vitro* dipeptidyl peptidase IV (DPP-IV) inhibitory and antioxidant properties

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ABSTRACT

The potential of quinoa to act as a source of dipeptidyl peptidase IV (DPP-IV) inhibitory and antioxidant peptides was studied. A quinoa protein isolate (QPI) with a purity of 40.73 ± 0.90% was prepared. The QPI was hydrolysed at 50 °C for 3 h with two enzyme preparations: papain (P) and a microbial papain-like enzyme (PL) to yield quinoa protein hydrolysates (QPHs). The hydrolysates were evaluated for their DPP-IV inhibitory and oxygen radical absorbance capacity (ORAC) activities. Protein hydrolysis was observed in the QPI control, possibly due to the activity of quinoa endogenous proteinases. The QPI control had significantly higher DPP-IV half maximal inhibitory concentrations (IC_{50}) and lower ORAC values than QPH-P and QPH-PL ($P < 0.05$). Both QPH-P and QPH-PL had similar DPP-IV IC_{50} and ORAC values. QPH-P had a DPP-IV IC_{50} value of 0.88 ± 0.05 mg mL^{-1} and an ORAC activity of 501.60 ± 77.34 μmol Trolox equivalent (T.E.) g^{-1}. To our understanding, this is the first study demonstrating the *in vitro* DPP-IV inhibitory properties of quinoa protein hydrolysates. QPHs may have potential as functional ingredients with serum glucose lowering properties.

1. Introduction

Due to the increasing prevalence of diabetes worldwide, the investigation of natural strategies to slow down the progress of this disease is a subject of interest to the scientific community. It has been suggested that natural components originating from foods can affect different biomarkers of type 2 diabetes (T2D). Among these, amino acids, peptides and food-derived proteins have been shown to affect serum glucose levels in normoglycaemic and T2D subjects (Manders et al., 2014). Although milk proteins appear to be one of the most studied substrates for the generation of insulinotropic components, selected studies have also demonstrated the benefit of ingesting plant proteins or plant protein hydrolysates in the regulation of serum glucose in humans (Méric et al., 2014). The antidiabetic properties of dietary proteins and peptides have been attributed to their direct insulinotropic properties or to the inhibition of metabolic enzymes such as dipeptidyl peptidase IV (DPP-IV) or α-glucosidase (Lacroix and Li-Chan, 2013; Mojica et al., 2015).

DPP-IV is responsible for the degradation of the incretin hormones such as glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Cleavage of the incretins by DPP-IV leads to a diminution of insulin secretion in pancreatic beta cells, in the post prandial phase (Juillerat-Jeanneret, 2014). DPP-IV inhibitory drugs, or gliptins, are currently being used for the treatment of T2D. DPP-IV inhibitors are also naturally found within

Abbreviations: AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; ACE, angiotensin converting enzyme; ACN, acetonitrile; AN, free amino group content; ANOVA, analysis of variance; BCA, bicinchoninic acid; BSA, bovine serum albumin; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPP-IV, dipeptidyl peptidase IV; DPP-IV PI, DPP-IV inhibitory potency index; E:S, enzyme to substrate ratio; GIP, glucose dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GP-HPLC, gel permeation high-performance liquid chromatography; HCl, hydrochloric acid; HPLC, high performance liquid chromatography; IC_{50}, half maximal inhibitory concentration; NaOH, sodium hydroxide; ORAC, oxygen radical absorbance capacity; P, papain; PL, papain-like enzyme; QPH, quinoa protein hydrolysate; QPH-P, QPH obtained with P; QPH-PL, QPH obtained with the PL; QPI, quinoa protein isolates; RP-HPLC, reverse-phase high-performance liquid chromatography; RuBisCo, ribulose-1,5-bisphosphate carboxylase/oxygenase; SD, standard deviation; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; TCA, trichloroacetic acid; T.E., Trolox equivalent; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TRIS, tris(hydroxymethyl)aminomethane; T2D, type 2 diabetes.

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a wide range of dietary proteins in the format of peptide fragments as demonstrated *in silico* (Lacroix and Li-Chan, 2012; Nongonierma and FitzGerald, 2014). These DPP-IV inhibitory peptides may be released during the enzymatic digestion of food proteins.

A relatively high oxidative status is generally found in individuals suffering from T2D as a consequence of the onset of secondary diseases including cardiovascular and renal complications (Hayden and Tyagi, 2001). Several studies have demonstrated that specific peptides from foods display an antioxidant activity *in vitro*. This can be seen through the scavenging of free radicals (Di Pierro et al., 2014; Nongonierma and FitzGerald, 2013) or through the inhibition/activation of certain pro- or anti-oxidative metabolic enzymes (Nongonierma and FitzGerald, 2012; O'Keeffe and FitzGerald, 2014). However, to date, a clear relationship between the consumption of dietary antioxidants and a reduction of *in vivo* oxidative status has not been established (Lacroix and Li-Chan, 2014).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal originating from South America which has gained increasing interest in other regions of the world over the past number years. This is linked with its high protein content and a balanced amino acid profile. It has been reported that quinoa contains higher content of proteins than other dietary grains such as wheat, rice, maize, oat and barley (González Martín et al., 2014). It is also becoming popular as a gluten-free grain. Only a restricted number of studies have demonstrated that quinoa potentially contains bioactive peptides. To date, it appears that quinoa peptides have mainly been studied for their *in vitro* angiotensin converting enzyme (ACE) inhibitory and antioxidant properties (Aluko and Monu, 2003). Recently, an *in silico* study has shown that quinoa proteins contain previously identified DPP-IV inhibitory peptides. A model was used to rank dietary proteins in terms of their DPP-IV inhibitory potency index (DPP-IV PI). It was shown that the large ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) chain from quinoa had a higher DPP-IV PI ($2.44 \cdot 10^{-6}$ μM^{-1} g^{-1}) than selected milk proteins such as bovine serum albumin (BSA) and α_{s2}-casein (0.93 and $1.93 \cdot 10^{-6}$ μM^{-1} g^{-1}, respectively) (Nongonierma and FitzGerald, 2014). This suggests that quinoa protein hydrolysates may have potential as a source of DPP-IV inhibitory peptides.

To our knowledge, no studies to date have shown that quinoa protein hydrolysates contain DPP-IV inhibitory properties. Therefore, the aim of this study was to generate quinoa protein hydrolysates which could inhibit DPP-IV. This was achieved by preparing a quinoa protein isolate (QPI). The QPI was hydrolysed with two food-grade enzymatic preparations. The peptide profiles of the resulting hydrolysates were then analysed. Finally, the samples were tested *in vitro* for their DPP-IV inhibitory and also for their antioxidant properties.

2. Materials and methods

2.1. Reagents

Organic Real quinoa seeds from Priméal (Paugres, France) containing 12.8% (*w*/*w*) protein were purchased in a local store (Limerick, Ireland). Trifluoroacetic acid (TFA), trichloroacetic acid (TCA), tris(hydroxymethyl)aminomethane (TRIS), azocasein, sodium phosphate monobasic, sodium phosphate dibasic, Gly-Pro-pNA, Leu, diprotin A (Ile-Pro-Ile), Trolox, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) radical, porcine DPP-IV ($\geq$10 U mg^{-1} protein) were obtained from Sigma Aldrich (Dublin, Ireland). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) was from Pierce Biotechnology (Medical Supply, Dublin, Ireland). Asp-Glu and Leu-Trp-Met-Arg were from Bachem (Bubendorf, Switzerland). Hydrochloric acid (HCl), sodium hydroxide (NaOH), high performance liquid chromatography (HPLC) grade water and acetonitrile (ACN) were from VWR (Dublin, Ireland). All other chemicals were of analytical grade and obtained from Sigma Aldrich.

2.2. Quinoa protein isolates (QPI)

QPI was prepared according to the method described by Aluko and Monu (2003) with modifications. Briefly, the quinoa seeds (300 g) were soaked for 60 min in 900 mL of distilled water. The quinoa seeds were then rinsed three times with the same volume (900 mL) of distilled water to remove saponins. The grains were reduced to a puree with an Ultraturrax homogeniser (IKA, Staufen, Germany) set at 6500 rpm for 20 min at room temperature (25 °C). The mixture was further diluted in distilled water at a 1:1 (*w*/*w*) ratio. The pH was adjusted to 9.0 using 0.5 M NaOH to solubilise the proteins under continuous agitation for 60 min at room temperature. The sample was then centrifuged (10,000 *g*, 30 min, 4 °C, Sorvall RC-5, Fisher Scientific, Dublin, Ireland). The supernatant was retained and subsequently adjusted to pH 4.6 with 0.1 N HCl and then centrifuged (10,000 *g*, 30 min, 4 °C). The proteins collected in the pellet were resuspended in distilled water (1:1 (*w*/*w*)) and adjusted to pH 7.0 with 0.5 M NaOH. The QPI sample was freeze-dried (FreeZone 18L, Labconco, Kansas City, MO, USA) and stored at −20 °C until utilisation.

The protein content of the QPI was determined with the bicinchoninic acid (BCA) method using a micro BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Absorbance at 562 nm was determined using a plate reader (Biotek Synergy HT, Winoosky, VT, USA) controlled by Gen 5 software (Biotek) and protein content was estimated by reference to a standard curve with bovine serum albumin (BSA) in the range of 25–2000 μg mL^{-1}. All samples were analysed in triplicate. The extraction yield (Equation (1)) and purity (Equation (2)) of the QPI were calculated as follows:

$$Yield = \frac{mass\ of\ protein\ in\ the\ QPI}{initial\ mass\ of\ protein} \times 100 \quad (1)$$

$$Purity = \frac{mass\ of\ protein\ in\ the\ QPI}{mass\ of\ QPI} \times 100 \quad (2)$$

2.3. Determination of the general proteinase activity of the enzyme preparations using the azocasein assay

The azocasein assay was used to determine the general proteolytic activity as described by Kilcawley et al. (2002). Briefly, the enzyme preparations were diluted at 1 g L^{-1} in 50 mM phosphate buffer, pH 7.0. A volume of 100 μL of the enzyme solution was incubated at 37 °C for 30 min with 1 mL of a 0.5% (*w*/*v*) azocasein solution in the phosphate buffer. The reaction was terminated by the addition of 100 μL of 2 M TCA. The samples were then centrifuged at 21,255 *g* for 5 min (Hettich Universal 320R, Hettich, Tuttlingen, Germany). The supernatant (750 μL) was mixed with 250 μL of 0.5 M NaOH and the absorbance was determined at 440 nm (UV mini 1240 spectrophotometer, Shimadzu, Kyoto, Japan).

2.4. Enzymatic hydrolysis of the QPI

Hydrolysis was carried out essentially as described by Nongonierma and FitzGerald (2015), with modifications. The QPI was resuspended in distilled water at 25 g L^{-1} on a protein basis, adjusted to pH 7.0 with 0.5 M NaOH and allowed to hydrate for 30 min at 50 °C. Two different enzyme preparations were used to