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# Activity, structural features and *in silico* digestion of antidiabetic peptides

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ABSTRACT

Food antidiabetic peptides inhibit the enzymes involved in the regulation of the glycemic index (e.g. α-amylase, α-glucosidase and dipeptidyl peptidase-IV (DPP-IV)). This work reviews the antidiabetic peptide sequences reported in the literature, with activity confirmed by using synthetic peptides, and critically discusses their structural features. Moreover, it provides an overview of the potency of *in silico* analysis tools to predict the *in vitro* antidiabetic activity of DPP-IV-inhibitory peptides. In addition, the potential degradation of the most active peptides during digestion was evaluated *in silico*. Therefore, this work advances our understanding on the structure-activity relationship of antidiabetic peptides and provides new insights on their stability during digestion.

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by the combined effect of insufficient insulin production and insulin resistance (i.e., inability of the organism to react to the insulin action) (Axelsson et al., 2017). Diabetes is the result of a deficient absorption of glucose, which is provoked by both genetic and environmental factors, such as nutritional habits. Insulin administration is the main treatment available to date. However, it is injected subcutaneously and cannot be orally administered since the active compound is degraded by digestive juices, hindering its easy use for patients (Howard-Thompson, Khan, Jones, & George, 2018).

Alternatively, diabetes could be tackled by acting on the enzymes involved in the carbohydrate hydrolysis, such as the α-amylase and α-glucosidase (Mustad, Huynh, López-Pedrosa, Campoy, & Rueda, 2020). By inhibiting these enzymes, carbohydrate digestion is delayed and blood sugar level lowered (Lakshmana Senthil, Chandrasekaran, Arjun, & Anantharaman, 2019). Hence, inhibitors of α-glucosidase and α-amylase, have been considered first-line medications for the management of T2DM. These synthetic inhibitors (i.e., acarbose, miglitol and voglibose) do not cause hypoglycemia and have a high safety profile; but they cause undesired gastrointestinal side-effects, limiting their administration for a long period (Kaur et al., 2021; Scheen, 2003).

Research into the pathophysiology of T2DM has led to the introduction of new medication approaches like those based on the inhibition of dipeptidyl peptidase IV (DPP-IV) (Ahmad & Chowdhury, 2019). DPP-IV regulates the insulin secretion, glucagon synthesis and gastric emptying by rapid degradation of incretins (i.e., Glucagon-like peptide 1, GLP-1, and Gastric Inhibitory Peptide, GIP) (Kazakos, 2011; Lammi et al., 2018; Pais, Gribble, & Reimann, 2016). DPP-IV inhibitors are drugs able to limit the degradation of GLP-1 and GIP, and thus, increasing the half-life of incretins, enabling to achieve an adequate metabolic condition. They have a good general safety and tolerability profile and have demonstrated to be effective in improving glycemic control but may present risk of acute pancreatitis (Deacon, 2020).

Despite the availability of various drugs for diabetes treatment, extensive research is still being conducted in the hopes of discovering useful, naturally derived molecules, free of side effects and toxicity (Park & Jang, 2017). Bioactive peptides derived from animal and plant proteins have recently attracted significant attention due to their multifaceted health benefits (Antony & Vijayan, 2021). The most widely used technologies to produce bioactive peptides are microbial fermentation and enzymatic hydrolysis (Cruz-Casas et al., 2021). Enzymatic hydrolysis might release bioactive peptides and may improve technological aspects of the protein, facilitate the digestibility by increasing the available N-terminal sites and decrease the antigenicity by degrading the allergenic epitopes (Rivero Pino, Pérez Gálvez, Espejo Carpio, & Guadix, 2020).

Food-derived peptides have been proven to exert different biological activities (El-Sayed & Awad, 2019; Karami & Akbari-adergani, 2019). It

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has been demonstrated that their bioactivity is determined by the specific amino acid sequence and the relative number of certain residues (e.g., hydrophilic, hydrophobic, or aromatic) (Sánchez & Vázquez, 2017) within the peptide. Besides, they may be free from serious side-effects since they are obtained from food sources that have been safely consumed over the years (Daliri, Oh, & Lee, 2017). Bioactive peptide sequences usually range in length from two to twenty amino acids, although longer peptides have also been reported (Korhonen & Pihlanto, 2006). Peptides displaying antidiabetic activity have normally low molecular weight, whose concentration and further isolation from the crude protein hydrolysate is challenging. To this regard, bioinformatic analyses play an essential role in predicting and identifying potentially bioactive compounds in this area. Computer simulation (*in silico*) might be a useful tool to predict the potential of peptide sequences such as DPP-IV inhibitors at a low-cost, which may help identify the relationship between peptide structure and its function. Other *in silico* tools can conduct simulated digestion on protein or peptide sequences, which is useful to design a targeted-hydrolysis process or to ensure that the sequence of a given peptide is not degraded during gastrointestinal digestion (Barati et al., 2020).

Hence, the aim of this review was to critically discuss the activity, structural features and *in silico* digestion of previously reported antidiabetic peptides. Furthermore, the potential of *in silico* tools to predict the *in vitro* antidiabetic activity of peptides was presented.

## 2. α-Amylase inhibitory peptides

α-Amylase (EC 3.2.1.1) is a digestive enzyme, secreted from the salivary and pancreatin glands, that hydrolyzes α-1,4 glycosidic bonds of complex carbohydrates, both linear and branched, into oligosaccharides (Fig. 1A). α-Amylase is responsible for starch digestion, thus inhibiting its activity will reduce the glucose spike in postprandial hyperglycemia (Kaur et al., 2021).

Therefore, a literature review of the peptide sequences exhibiting α-amylase inhibitory activity by searching in Scopus and Pubmed databases (period between 2015 and March 2022) was carried out. The following keywords were used: "antidiabetic peptide", "α-amylase inhibitory peptide", "synthetic antidiabetic peptide" and "hydrolysis antidiabetic peptide". Only studies reporting experimental $IC_{50}$ value of the peptides identified were selected. Table 1 provides the complete list of α-amylase inhibitory peptides identified recently in literature, which were arranged by protein source and $IC_{50}$ (μM).

The total number of peptide sequences with available *in vitro* values of α-amylase inhibitory activity is limited in the literature, reporting only 18 sequences with experimental $IC_{50}$. Most of the sequences identified were obtained from plant substrates, reflecting the focus on sustainable protein sources such as plants and algae, which has been a trend in the last years. Although some studies based on animal protein sources were found, they have been disregarded due to their antiquity (Yu, Yin, Zhao, Liu, & Chen, 2012) or because no sequence identification was carried out (Liu, Wang, Peng, & Wang, 2013; Kumar, Shakila, & Jeyasekaran, 2019). Acarbose is the most common positive control referenced, with $IC_{50}$ values ranging from 100 to 774.47 μM (Xiong et al., 2020). This wide difference shows a lack of harmonization of the analysis, implying a possible misrepresentation in the results found and highlighting the need for standard methods.

$IC_{50}$ values for the peptides reported in literature range from 0.02 to 2000 μM (Table 1). Sequences presenting lower $IC_{50}$ values shared common attributes, which could be related to the hydrolysis procedure. The protease employed in the hydrolysis is a key parameter to release antidiabetic peptides. In this regard, most of the sequences reported were obtained with Protamex, individually or in combination with other proteases. Protamex is a broad-spectrum endo-protease which cleaves preferably hydrophobic and aromatic residues (i.e., L, F, and V among others) (Ustunol, 2015). Peptide FFRSKLLSDGAAAAKGALLPQYW had the lowest $IC_{50}$. It was obtained by hydrolysis of cumin seed protein with Protamex at pH 8 and 42.6 °C (Siow & Gan, 2016). Other studies (Esfandi, Seidu, Willmore, & Tsopmo, 2022; Ngoh & Gan, 2018) hydrolyzed pinto beans and oat meal at pH 7.5 and 50 °C with both Protamex and papain (Esfandi et al., 2022) —a cysteine protease which cleaves positively charged amino acids, such as A, K, and residues following F (Vatić, Mirković, Milošević, Jovčić, & Polović, 2020). The remaining proteases reported were both endopeptidases of broad specificity: Alcalase (Wang et al., 2020) and pepsin (Admassu, Gasmalla, Yang, & Zhao, 2018). Alcalase preferences hydrophobic and aromatic residues, while pepsin shows specificity towards the Ct of F and L (Ahn,

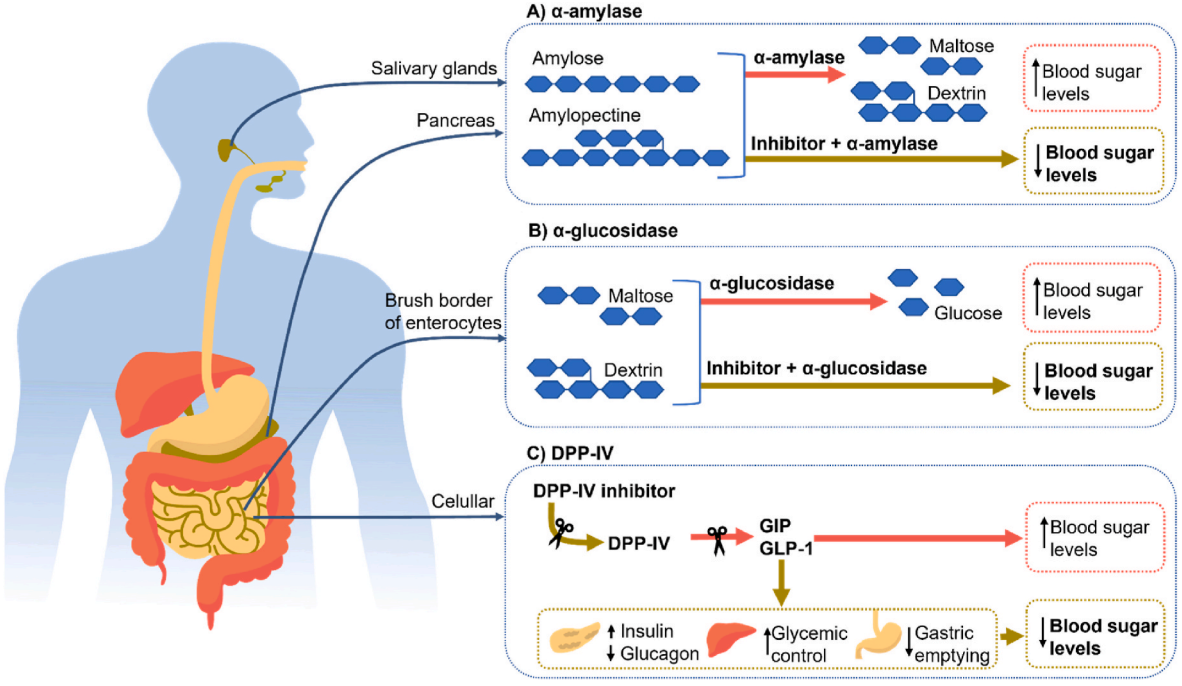


**Fig. 1.** Inhibition mechanisms of A) α-amylase, B) α-glucosidase and C) DPP-IV for the control of DMT2.

**Table 1**
Summary and structure analysis of α-amylase inhibiting peptides found in literature.

| Type | Source | Sequence | Enzymatic treatment | $IC_{50}$ (μM) | PCL | % Hydrophobic AA | L in Nt | P in Nt | pI | Net charge | Estimated solubility | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant | Cumin seed (*Cuminum cyminum*) | FFRSKLLSDGAAAAKGALLPQYW | Protamex | 0,02 | 23 | 60,87% | | | 10,20 | 2 | Poor | Siow and Gan (2016) |
| Plant | Cumin seed (*Cuminum cyminum*) | DPAQPNYPWTAVLVFRH | Protamex | 0,03 | 17 | 52,94% | | DP | 7,78 | 0.1 | Poor | Siow and Gan (2016) |
| Plant | Cumin seed (*Cuminum cyminum*) | RCMAFLLSDGAAAAQQLLPQYW | Protamex | 0,04 | 22 | 59,09% | | | 5,90 | −0.1 | Poor | Siow and Gan (2016) |
| Plant | Oat meal (*Avena sativa*) | YFDEQNEQFR | Papain & Protamex | 37,5 | 10 | 0,00% | | | 3,69 | −2 | Good | Esfandi et al. (2022) |
| Plant | Oat meal (*Avena sativa*) | NINAHSVVY | Papain & Protamex | 67,3 | 9 | 44,44% | | | 7,38 | 0.1 | Poor | Esfandi et al. (2022) |
| Plant | Oat meal (*Avena sativa*) | RALPIDVL | Papain & Protamex | 72,8 | 8 | 75,00% | | | 6,35 | 0 | Good | Esfandi et al. (2022) |
| Plant | Spirulina platensis (*Spirulina platensis*) | LRSELAAWSR | No hydrolysis | 264,0 | 10 | 50,00% | LR | | 10,68 | 1 | Good | (Hu et al., 2019) |
| Plant | Spirulina platensis (*Spirulina platensis*) | GVPMPNK | No hydrolysis | 318,3 | 7 | 71,43% | | | 10,12 | 1 | Good | (Hu et al., 2019) |
| Plant | Spirulina platensis (*Spirulina platensis*) | RNPFVFAPTLLTVAAR | No hydrolysis | 607,8 | 16 | 56,25% | | | 12,10 | 2 | Poor | (Hu et al., 2019) |
| Animal | Parmigiano-Reggiano Cheese | IPP | Ripening at 12–30 months | 763,5 | 3 | 100,00% | | IP | 13,10 | 0 | Poor | Martini et al. (2021) |
| Plant | Walnut (*Juglans mandshurica*) | LPLLR | Alcalase | 2000 | 5 | 80,00% | LP | LP | 10.84 | 1 | Poor | (Wang et al., 2020) |
| Seaweed | Red Seaweed (*Porphyra* spp) | GGSK | Pepsin | 2580 | 4 | 50,00% | | | 10.12 | 1 | Good | Admassu et al. (2018) |
| Seaweed | Red Seaweed (*Porphyra* spp) | ELS | Pepsin | 2620 | 3 | 33,33% | EL | | 1.01 | −1 | Good | Admassu et al. (2018) |
| Plant | Pinto bean (*Phaseolus vulgaris*) | LSSLEMGSLGALFVCM | Protamex | 10030 | 16 | 62,50% | LS | | 1,00 | −1.1 | Poor | Ngoh and Gan (2018) |
| Plant | Pinto bean (*Phaseolus vulgaris*) | PLPWGAGF | Protamex | 15730 | 8 | 87,50% | PL | PL | 4.15 | 0 | Poor | Ngoh and Gan (2018) |
| Plant | Pinto bean (*Phaseolus vulgaris*) | PLPLHMLP | Protamex | 15800 | 8 | 87,50% | PL | PL | 8.26 | 0.1 | Poor | Ngoh and Gan (2018) |
| Plant | Pinto bean (*Phaseolus vulgaris*) | PPHMGGP | Protamex | 19830 | 7 | 85,71% | | PP | 8.26 | 0.1 | Poor | Ngoh and Gan (2018) |
| Plant | Pinto bean (Phaseolus vulgaris) | PPHMLP | Protamex | 23330 | 6 | 83,33% | | PP | 8.26 | 0.1 | Poor | Ngoh and Gan (2018) |

$IC_{50}$ (μM): half maximal inhibitory activity, expressed in μM.
PCL: peptide chain length.
L in Nt: presence of leucine in the last two positions of the N-terminus.
P in Nt: presence of proline in the last two positions of the N-terminus.
pI: isoelectric point determined by Innovagen's peptide calculator PepCalc.
Net charge was determined at pH 7 by Innovagen's peptide calculator PepCalc.
Estimated solubility: solubility is estimated since Innovagen's tool does not take into consideration factors such as peptide concentration.

Cao, Yu, & Engen, 2013; Tang et al., 2018). Peptides released by these enzymes showed low inhibitory activity ($IC_{50}$ = 2000–2620 μM).

Sequences LRSELAAWSR, GVPMPNK and RNPFVFAPTLLTVAAR were extracted from *Spirulina platensis* by ultrasound coupled with subcritical water (USW) technology (Hu, Fan, Qi, & Zhang, 2019). Microbial fermentation was used to obtain the tripeptide IPP from Parmigiano-Reggiano Cheese (Martini, Solieri, Cattivelli, Pizzamiglio, & Tagliazucchi, 2021). These peptides presented $IC_{50}$ values of 264.0–763.5 μM.

In general, there is little information on the production of α-amylase inhibitory peptides by enzymatic hydrolysis. Further work is needed for the isolation of active sequences by enzymatic processing, employing

new proteases of different specificity.

In attempt to gain more insights into the activity of α-amylase inhibitory peptides, the most frequently reported structural features for bioactive peptides were studied and linked to their activity, namely: their average peptide chain length (PCL), number of hydrophobic residues, ratio of hydrophobic to total residues, pI, presence of L and P in the last two positions at the Nt (L in Nt, P in Nt) and estimated solubility (Ngoh & Gan, 2018) (Table 1).

Identified sequences have a wide PCL distribution, 3–23 residues, with ~70% of them being 3–10 residues long. Hydrophobic interactions and hydrogen bonds also play a large part in substrate binding to α-amylase (Tysoe et al., 2016). All peptides, except for YFDEQNEQFR (Esfandi et al., 2022), contained over 44% of hydrophobic amino acids. Furthermore, presence of these residues (i.e., P and L) at the Nt of the sequences has been proposed as an important characteristic of α-amylase inhibitors (Ngoh & Gan, 2018). The Nt last two positions were studied, finding that 6 out of the 18 peptides contained L and 7 contained P. Other important identifying factor of these peptides is the presence of F residues at either end (Ngoh & Gan, 2016), however only 2 of the peptides met this characteristic.

The pI, net charge and estimated solubility of the peptides were also calculated. pI values of 5-6 have been reported to lower the degradation rate of the peptides by deamidation (Keservani, Sharma, & Jarouliya, 2015). Twelve of the peptides identified were estimated *in silico* to have poor solubility in water, adding difficulty to its use as a functional ingredient, due to its technological limitations. This low solubility is not only affected by pI but also by the hydrophobic amino acids content (A, V, I, L, M, Y, W, G, and P). Indeed, peptides with poor solubility contained at least 50% of hydrophobic amino acids. Out of 18 peptides, 11 had positive net charge at pH 7 (Table 1), which could positively affect their absorption by transcytosis transport according to previous studies on cell models (Amigo & Hernández-Ledesma, 2020).

The most active peptide identified had a PCL of 23 residues (Siow & Gan, 2016), making it the longest sequence. Its hydrophobic amino acids content was ca. 61% and did not present L or P residues at the Nt. Nonetheless, there are only a few studies evaluating the kinetics and inhibition mode of α-amylase inhibitory peptides. Among the few studies, Admassu et al. (Admassu et al., 2018) investigated the α-amylase inhibition mechanism for red seaweed peptides, using the Lineweaver-Burk double reciprocal of the velocity-substrate plot, observing a near non-competitive mode of inhibition. The authors reported that the peptides bounded to the allosteric site of the enzyme leading to conformation changes in the enzyme, which did not allow the bind of the substrate to the enzyme or inhibited the enzyme activity to convert substrate into product. Using the same approach, Ngoh et al. (Ngoh, Tye, & Gan, 2017) studied the α-amylase inhibition mechanism of five Pinto bean peptides, revealing that three of them displayed uncompetitive inhibition when binding to the (α-amylase)-starch complex. This binding altered the α-amylase structure and resulted in the detachment of starch from the complex, thereby preventing its hydrolysis. Interestingly, the remaining two peptides exhibited an unconventional inhibition mechanism, as they required a higher concentration of substrate for binding to either the substrate or the enzyme, which resulted in the inability of starch and enzyme to bind, thus leading to the inhibition. Another study conducted on soybean protein investigated the inhibition mechanism for four peptides (Awosika & Aluko, 2019). Three of them exhibited mixed inhibition, displaying both competitive and uncompetitive modes, indicating that they can bind to both the free enzyme and the enzyme-substrate complex. These two modes of inhibition could act synergistically if two peptides bind simultaneously. Another peptide identified in soybeans was a pure uncompetitive inhibitor, which specifically binds to non-carbohydrate binding sites rather than the active site. Therefore, further analysis identifying and evaluating peptides experimentally is needed to draw conclusions about the structure-activity relationship of α-amylase inhibitory peptides. The lack of research in this area can be explained by the lower physiological relevance of these inhibitors compared to α-glucosidase and DPP-IV inhibitory peptides.

## 3. α-Glucosidase inhibitory peptides

α-Glucosidase (EC 3.2.1.20), which is found in the epithelial mucosa of the small intestine (brush border of the enterocytes), degrades the oligosaccharides produced by α-amylase, releasing free glucose molecules from terminal, non-reducing (1–4)-linked α-D glucose residues (Fig. 1B). Hence, inhibition of α-glucosidase allows reducing the release of glucose from ingested carbohydrates and its absorption, which leads to a decrease of postprandial blood glucose levels (Hossain, Das, Ghosh, & Sil, 2020).

The sequences of α-glucosidase inhibitory peptides identified in the literature were studied, following the search criteria previously mentioned and including "α-glucosidase inhibitory peptide" as keyword (Table 2). According to the literature research, α-glucosidase inhibitory peptides were isolated from both animal and plant sources. It is worth mentioning that, among the animal sources, one of the most used substrates were insects, mainly mealworm and desert locust (Zhang et al., 2016; Zielińska, Karaś, Baraniak, & Jakubczyk, 2020). Interest in insects as a source of bioactive peptides dated back to 2005 (Vercruysse, Smagghe, Herregods, & Van Camp, 2005), although their use for antidiabetic peptides has started recently (Nongonierma & FitzGerald, 2017; Rivero-Pino et al., 2020).

Acarbose is the reference control most frequently reported in literature but, inconsistencies were identified concerning the $IC_{50}$ values, which ranged from 3.34 μM (Sulistiyani, Safithri, & Sari, 2016) to 2338.90 μM (Zhao, Su, Mao, & Zhang, 2020). This is in line with the data found for α-amylase inhibitory peptides. Although the reason for these differences could not be reliably determined, it can be estimated that they are due to the use of different methods to perform the inhibition analysis, sources of acarbose and enzyme/substrate ratios. This non-conformity in the performance of analysis should be considered, since the α-glucosidase inhibitory peptides identified present a wide range of $IC_{50}$, from 7.93 to 2000 μM (Table 2). This implies a difference of 700-fold between the lowest and the highest value, which might be related not only to real differences in peptides activity but also to lack of harmonization on the methods.

Most of the α-glucosidase inhibitory peptides identified so far in the literature were produced enzymatically by digestive proteases (i.e., trypsin, chymotrypsin, and pepsin) (Ibrahim, Bester, Neitz, & Gaspar, 2018b). Trypsin is a very specific protease, cleaving only R and K residues, whereas chymotrypsin preferentially recognizes bulky aromatic residues such as F, Y, and W (Olsen, Ong, & Mann, 2004). Pepsin shows a narrower specificity, cleaving after F and L residues (Ahn et al., 2013). The hydrolysis conditions, including those set for the *in silico* simulations, were physiological conditions. The peptides released presented $IC_{50}$ values of 7.93–1215.42 μM. The most active peptide sequence identified–FDPFPK–was obtained by simulating oral, gastric, and intestinal digestion (Zielińska et al., 2020).

Other authors produced α-glucosidase inhibitory peptides employing different commercial proteases. For instance, two recent works report inhibitory peptides obtained with Alcalase at pH 9 and 50 °C, reporting three peptides from soybean protein with $IC_{50}$ values ranging 162.29–237.43 μM (Wang et al., 2020; Wang et al., 2019), and one peptide from walnut seeds with moderate inhibitory potency, 2000 μM (Wang et al., 2020; Wang et al., 2019). Other studies hydrolyzed soft-shelled turtle employing a variety of commercial proteases (i.e., Alcalase, Flavourzyme, papain and neutrase) at optimal conditions (Qiu et al., 2021). Papain hydrolysates were fractionated by ultrafiltration and reverse chromatography, allowing the identification of three inhibitory peptides (HNKPEVEVR, ARDASVLK, SGTLLHK), which presented the highest α-glucosidase inhibitory activity with $IC_{50}$ values of 162.29–237.43 μM.

Several studies used alternative technologies to produce inhibitory

**Table 2**
Summary and structure analysis of α-glucosidase inhibiting peptides.

| Type | Source | Sequence | Enzymatic treatment | $IC_{50}$ (μM) | PCL | % Hydrophobic AA | S in Nt | T in Nt | Y in Nt | P in Ct | A in Ct | M in Ct | pI | Net charge | Estimated solubility | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Desert locus (*Schistocerca gregaria*) | FDPFPK | Gastrointestinal digestion | 7,93 | 6 | 33,33% | | | | PK | | | 6,39 | 0 | Good | Zielińska et al. (2020) |
| Animal | Mealworm (*Tenebrio molitor*) | AAAPVAVAK | Gastrointestinal digestion | 13,70 | 9 | 88,89% | | | | | AK | | 10,19 | 1 | Poor | Zielińska et al. (2020) |
| Animal | Desert locus (*Schistocerca gregaria*) | AIGVGAIER | Gastrointestinal digestion | 14,73 | 10 | 50,00% | | | | | | | 6,93 | 0 | Good | Zielińska et al. (2020) |
| Plant | Almond oil manufacture residue (*Prunus dulcis*) | WH | Prote Ax and protease M | 17,03 | 2 | 50,00% | | | | | | | 7,69 | 0.1 | Poor | Gu, Gao, Hou, Li, and Fu (2020) |
| Animal | Silkworm pupae (*Bombyx mori*) | SQSPA | *In silico* digestion | 20,00 | 5 | 40,00% | SQ | | | PA | PA | | 3,36 | 0 | Good | (Zhang et al., 2016) |
| Plant | Soybean protein (*Glycine max*) | GSR | Trypsin | 20,40 | 3 | 0,00% | GS | | | | | | 10,84 | 1 | Good | Jiang et al. (2018) |
| Plant | Changium Root (*Changii Radix*) | KVIISAPSKDAPMF | Simulated gastrointestinal digestion | 21,28 | 15 | 53,33% | | | | | | | 9,74 | 1 | Good | (Liu, Chen, & Li, 2021) |
| Animal | Desert locus (*Schistocerca gregaria*) | GKDAVIV | Gastrointestinal digestion | 22,74 | 7 | 57,14% | | | | | | | 6,63 | 0 | Good | Zielińska et al. (2020) |
| Animal | Cricket (*Grylloides sigillatus*) | KVEGDLK | Gastrointestinal digestion | 23,31 | 7 | 28,57% | | | | | | | 6,71 | 0 | Good | Zielińska et al. (2020) |
| Plant | Almond oil manufacture residue (*Prunus dulcis*) | WS | Prote Ax and protease M | 24,71 | 2 | 50,00% | WS | | | | | | 3,61 | 0 | Poor | Gu et al. (2020) |
| Animal | Mealworm (*Tenebrio molitor*) | NYVADGLG | Gastrointestinal digestion | 25,21 | 9 | 33,33% | | | NY | | | | 0,68 | −1 | Poor | Zielińska et al. (2020) |
| Animal | Mealworm (*Tenebrio molitor*) | AGDDAPR | Gastrointestinal digestion | 27,77 | 7 | 42,86% | | | | PR | | | 3,71 | −1 | Good | Zielińska et al. (2020) |
| Animal | Cricket (*Grylloides sigillatus*) | IIAPPER | Gastrointestinal digestion | 28,75 | 8 | 62,50% | | | | | | | 6,87 | 0 | Good | Zielińska et al. (2020) |
| Plant | Dark tea protein (*Camellia sinensis*) | VVDLVFFAAAK | No hydrolysis | 33,93 | 11 | 63,64% | | | | | AK | | 6,60 | 0 | Poor | Zhao et al. (2020) |
| Animal | Soft-shelled turtle egg (*Pelodiscus sinensis*) | HNKPEVEVR | Papain | 56,00 | 9 | 33,33% | | | | | | | 7,56 | 0.1 | Good | Qiu et al. (2021) |
| Animal | Cricket (*Grylloides sigillatus*) | LAPSTIK | Gastrointestinal digestion | 62,55 | 7 | 57,14% | | | | | | | 10,12 | 1 | Good | Zielińska et al. (2020) |
| Animal | Silkworm pupae (*Bombyx mori*) | QPGR | *In silico* digestion | 65,80 | 4 | 25,00% | | | | | | | 10,55 | 1 | Good | (Zhang et al., 2016) |
| Plant | Spirulina platensis (*Spirulina platensis*) | RNPFVFAPTLLTVAAR | No hydrolysis | 92,78 | 17 | 52,94% | | | | | | | 12,10 | 2 | Poor | (Hu et al., 2019) |
| Plant | Quinoa (*Chenopodium quinoa*) | IQAEGGLT | Simulated digestion | 109,48 | 8 | 37,50% | | | | | | | 0,97 | −1 | Poor | Vilcacundo, Martínez-Villaluenga, and Hernández-Ledesma (2017) |
| Plant | Spirulina platensis (*Spirulina platensis*) | LRSELAAWSR | No hydrolysis | 112,96 | 10 | 50,00% | | | | | | | 10,68 | 1 | Good | (Hu et al., 2019) |
| Plant | Soybean protein (*Glycine max*) | WLRL | Alkaline proteinase | 162,29 | 4 | 75,00% | | | | | | | 10,72 | 1 | Poor | (Wang et al., 2019) |

(*continued on next page*)

**Table 2** (*continued*)

| Type | Source | Sequence | Enzymatic treatment | $IC_{50}$ (μM) | PCL | % Hydrophobic AA | S in Nt | T in Nt | Y in Nt | P in Ct | A in Ct | M in Ct | pI | Net charge | Estimated solubility | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant | Soybean protein (*Glycine max*) | SWLRL | Alkaline proteinase | 182,05 | 5 | 60,00% | SW | | | | | | 10,57 | 1 | Poor | (Wang et al., 2019) |
| Animal | Soft-shelled turtle egg (*Pelodiscus sinensis*) | ARDASVLK | Papain | 195,00 | 8 | 50,00% | | | | | | | 10,18 | 1 | Good | Qiu et al. (2021) |
| Plant | Spirulina platensis (*Spirulina platensis*) | GVPMPNK | No hydrolysis | 204,18 | 8 | 50,00% | | | | | | | 10,12 | 1 | Good | (Hu et al., 2019) |
| Animal | Silkworm pupae (*Bombyx mori*) | NSPR | *In silico* digestion | 205,00 | 4 | 25,00% | NS | | | PR | | | 10,42 | 1 | Good | (Zhang et al., 2016) |
| Plant | Soybean protein (*Glycine max*) | LLPLPVLK | Alkaline proteinase | 237,43 | 8 | 87,50% | | | | | | | 10,12 | 1 | Poor | (Wang et al., 2019) |
| Animal | Soft-shelled turtle egg (*Pelodiscus sinensis*) | SGTLLHK | Papain | 289,00 | 7 | 28,57% | SG | | | | | | 9,86 | 1.1 | Good | (Qiu et al., 2021)( |
| Animal | Eggyolk protein by-product (*Gallus domesticus*) | VTGRFAGHPAAQ | Pepsin | 301,73 | 12 | 41,67% | | VT | | | AQ | | 10,81 | 1.1 | Poor | Zambrowicz et al. (2015) |
| Animal | Eggyolk protein by-product (*Gallus domesticus*) | YINQMPQKSREA | Pepsin | 310,31 | 12 | 33,33% | | | YI | | EA | | 9,49 | 1 | Good | Zambrowicz et al. (2015) |
| Plant | Soybean protein (*Glycine max*) | EAK | Trypsin | 520,20 | 3 | 33,33% | | | | | AK | | 6,85 | 0 | Good | Jiang et al. (2018) |
| Plant | Changium Root (*Changii Radix*) | SQHISTAGMEASGTSNMKF | Simulated digestion | 529,74 | 20 | 25,00% | SQ | | | | | | 7,54 | 0.1 | Poor | (Liu et al., 2021) |
| Plant | Dark tea protein (*Camellia sinensis*) | TAELLPR | No hydrolysis | 538,17 | 7 | 57,14% | | TA | | PR | | | 6,55 | 0 | Good | (Zhao et al., 2020)) |
| Animal | Silkworm pupae (*Bombyx mori*) | QPPT | *In silico* digestion | 560,00 | 4 | 50,00% | | | | PT | | | 3,40 | 0 | Poor | (Zhang et al., 2016) |
| Plant | Dark tea protein (*Camellia sinensis*) | CGKKFVR | No hydrolysis | 621,27 | 7 | 14,29% | | | | | | | 10,82 | 2.9 | Good | Zhao et al. (2020) |
| Plant | Dark tea protein (Camellia sinensis) | AVPANLVDLNVPALLK | No hydrolysis | 625,38 | 16 | 75,00% | | | | | | | 6,69 | 0 | Poor | (Zhao et al., 2020)( |
| Animal | Parmigiano-Reggiano Cheese | IPP | Ripening at 12–30 months | 764,50 | 3 | 100,00% | | | | PP | | | 3,83 | 0 | Poor | Martini et al. (2021) |
| Plant | Changium Root (*Changii Radix*) | STFQQMW | Simulated digestion | 1190,94 | 8 | 25,00% | ST | ST | | | | | 3,34 | 0 | Poor | (Liu et al., 2021) |
| Animal | Eggyolk protein by-product (*Gallus domesticus*) | YINQMPQKSRE | Pepsin | 1215,42 | 11 | 27,27% | | | YI | | | | 9,49 | 1 | Good | Zambrowicz et al. (2015) |
| Plant | Walnut (*Juglans mandshurica*) | LPLLR | Alcalase | 2000,00 | 5 | 80,00% | | | | | | | 10,84 | 1 | Poor | (Wang et al., 2020) |

$IC_{50}$ (μM): half maximal inhibitory activity, expressed in μM.
PCL: peptide chain length.
S in Nt: presence of serine in the last two positions of the N-terminus.
T in Nt: presence of threonine in the last two positions of the N-terminus.
Y in Nt: presence of tyrosine in the last two positions of the N-terminus.
T in Ct: presence of proline in the last two positions of the C-terminus.
A in Ct: presence of alanine in the last two positions of the C-terminus.
M in Ct: presence of methionine in the last two positions of the C-terminus.
pI: isoelectric point determined by Innovagen's peptide calculator PepCalc.
Net charge was determined at pH 7 by Innovagen's peptide calculator PepCalc.
Estimated solubility: solubility is estimated since Innovagen's tool does not take into consideration factors such as peptide concentration.

**Table 3**
Summary and structure analysis of DPP-IV inhibiting peptides.

| Type | Source | Sequence | Enzymatic treatment | $IC_{50}$ (μM) | PCL | Score (DPP-IVi) | Stack DPPIV | % Hydrophobic AA | A in Nt | P in Nt | P in Nt* | F in Ct | W in Ct | Y in Ct | pI | Net charge | Estimated solubility | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Velvet aqueous extract (*Cervus elaphus*) | GPAGPQGPR | Gastric-pancreatic digestion | 0,51 | 9 | 320,88 | 0.47 | 77,78% | | GP | GPAG | | | | 10,84 | 0 | Good | (Yu et al., 2017) |
| Animal | Velvet aqueous extract (*Cervus elaphus*) | PPGLPGSPGQ | Gastric-pancreatic digestion | 0,55 | 10 | 342,78 | 0.62 | 80,00% | | PP | PPGL | | | | 4,15 | 0 | Poor | (Yu et al., 2017) |
| Animal | Velvet aqueous extract (*Cervus elaphus*) | LPQPPQE | Gastric-pancreatic digestion | 0,97 | 7 | 439,50 | 0.92 | 57,14% | | LP | LPQP | | | | 1,00 | −1 | Good | (Yu et al., 2017) |
| Animal | Velvet aqueous extract (*Cervus elaphus*) | LPPLTAD | Gastric-pancreatic digestion | 1,67 | 7 | 369,67 | 0.84 | 71,43% | | LP | LPPL | | | | 0,88 | −1 | Poor | (Yu et al., 2017) |
| Plant | Picrorhiza kurroa (*Picrorhiza kurrooa*) | ASGLCPEEAVPRR | Trypsin | 2,20 | 14 | 247,33 | 0.06 | 50,00% | AS | | | | | | 6,29 | −0.1 | Good | Thakur et al. (2021) |
| Animal | Boarfish (*Capros aper*) | IPV | Alcalase and Flavourzyme | 5.61 | 3 | 500,00 | 1 | 100,00% | | IP | IPV | | | | 3.66 | 0 | Poor | (Harnedy-Rothwell et al., 2020) |
| Animal | Camel whey protein (*Camelus dromedarius*) | VPV | Trypsin | 6,6 | 3 | 531,00 | 1 | 100,00% | | VP | VPV | | | | 3,63 | 0 | Poor | Nongonierma et al. (2019) |
| Plant | Sorghum bicolor seed protein (*Sorghum bicolor* L.) | QLRDIVDK | *In silico* gastrointestinal digestion | 8,55 | 9 | 229,57 | 0.27 | 33,33% | | | | | | | 6,50 | 0 | Good | Majid et al. (2022) |
| Animal | Boarfish (*Capros aper*) | IPVDM | Alcalase and Flavourzyme | 21,72 | 5 | 397,25 | 0.75 | 80,00% | | IP | IPVD | | | | 0,78 | −1 | Good | Harnedy-Rothwell et al. (2020) |
| Plant | Quinoa (*Chenopodium quinoa*) | HPF | *In silico* bromelain | 34,31 | 3 | 519,50 | 0.97 | 33,33% | | HP | HPF | PF | | | 7,56 | 0.1 | Poor | Guo et al. (2020) |
| Animal | Boarfish (*Capros aper*) | APIT | Alcalase and Flavourzyme | 34,73 | 4 | 401,33 | 0.95 | 75,00% | AP | AP | APIT | | | | 3,76 | 0 | Poor | Harnedy-Rothwell et al. (2020) |
| Animal | Camel whey protein (*Camelus dromedarius*) | YPI | Trypsin | 35,00 | 3 | 493,50 | 0.91 | 100,00% | | YP | YPI | | | | 3,37 | 0 | Poor | (Nongonierma, Cadamuro, le Gouic et al., 2019) |
| Plant | Brewers' spent grain (*Hordeum vulgare*) | IPVP | Alcalase and Flavourzyme | 38,67 | 4 | 493,33 | 0.69 | 100,00% | | IP | IPVP | | | | 3,83 | 0 | Poor | Cermeño et al. (2019) |
| Animal | Boarfish (*Capros aper*) | VPTP | Alcalase and Flavourzyme | 38,93 | 4 | 510,00 | 0.93 | 75,00% | | VP | VPTP | | | | 3,78 | 0 | Poor | Harnedy-Rothwell et al. (2020) |
| Animal | Discarded shrimp head (*Penaeus vannamei*) | YPGE | Animal protease | 40,9 | 4 | 401,00 | 0.95 | 75,00% | | YP | YPGE | | | | 1,00 | −1 | Good | Xiang et al. (2021) |
| Animal | Casein | VPYPQ | Neutrase | 41,45 | 6 | 462,25 | 0.45 | 66,67% | | VP | VPYP | | | | 3,62 | 0 | Poor | Zheng et al. (2019) |
| Animal | Atlantic salmon (*Salmo salar*) | GPGA | Flavourzyme | 41,9 | 4 | 344,67 | 0.81 | 100,00% | | GP | GPGA | | | | 3,63 | 0 | Poor | Li-Chan et al. (2012) |
| Animal | Whey protein isolate | LKPTPEGDLE | Thermoase PC10F | 42 | 10 | 302,56 | 0.7 | 50,00% | | | LKPT | | | | 3,69 | −2 | Good | Lacroix, Meng, Cheung, and Li-Chan (2016) |
| Plant | Dulse (*Palmaria palmata*) | ILAP | Corolase PP | 43,4 | 4 | 402,00 | 0.96 | 100,00% | | | ILAP | | | | 3,83 | 0 | Poor | (Harnedy et al., 2015) |

(*continued on next page*)

**Table 3** (*continued*)

| Type | Source | Sequence | Enzymatic treatment | $IC_{50}$ (μM) | PCL | Score (DPP-IVi) | Stack DPPIV | % Hydrophobic AA | A in Nt | P in Nt | P in Nt* | F in Ct | W in Ct | Y in Ct | pI | Net charge | Estimated solubility | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Whey protein concentrate rich in β-lactoglobulin | IPAVF | Trypsin | 44,70 | 5 | 371,50 | 0.8 | 80,00% | | IP | IPAV | VF | | | 3,71 | 0 | Poor | Silveira, Martínez-Maqueda, Recio, and Hernández-Ledesma (2013) |
| Plant | Brewers' spent grain (*Hordeum vulgare*) | LPIA | Alcalase and Flavourzyme | 45,07 | 4 | 402,00 | 1 | 100,00% | | LP | LPIA | | | | 3,63 | 0 | Poor | Cermeño et al. (2019) |
| Animal | Boarfish (*Capros aper*) | GPIN | Alcalase and Flavourzyme | 48,96 | 4 | 391,33 | 0.52 | 75,00% | | GP | GPIN | | | | 3,71 | 0 | Poor | Harnedy-Rothwell et al. (2020) |
| Animal | Parmigiano-Reggiano Cheese | APFPE | Ripening at different times | 49,50 | 5 | 430,50 | 0.87 | 60,00% | AP | AP | APFP | | | | 1,00 | −1 | Good | Martini et al. (2021) |
| Animal | Atlantic salmon (*Salmo salar*) | GPAE | Flavourzyme | 49,60 | 4 | 380,33 | 0.53 | 75,00% | | GP | GPAE | | | | 1,00 | −1 | Good | Li-Chan et al. (2012) |
| Animal | Boarfish (*Capros aper*) | LPVYD | Alcalase and Flavourzyme | 51,36 | 5 | 382,75 | 0.55 | 80,00% | | LP | LPVY | | | YD | 0,88 | −1 | Poor | Harnedy-Rothwell et al. (2020) |
| Plant | Brewers' spent grain (*Hordeum vulgare*) | IPY | Alcalase and Flavourzyme | 52,15 | 3 | 493,50 | 0.91 | 100,00% | | IP | IPY | | | PY | 3,62 | 0 | Poor | Cermeño et al. (2019) |
| Plant | Rapeseed napin (*Brassica napus*) | IPQVS | Alcalase and trypsin | 52,16 | 5 | 344,25 | 0.45 | 60,00% | | IP | IPQV | | | | 3,73 | 0 | Poor | (Xu et al., 2019) |
| Animal | Boarfish (*Capros aper*) | LPVDM | Alcalase and Flavourzyme | 53,50 | 5 | 410,25 | 0.67 | 80,00% | | LP | LPVD | | | | 0,78 | −1 | Good | Harnedy-Rothwell et al. (2020) |
| Plant | Dulse (*Palmaria palmata*) | LLAP | Corolase PP | 53,74 | 4 | 419,33 | 0.98 | 100,00% | | | LLAP | | | | 3,82 | 0 | Poor | Harnedy et al. (2015) |
| Plant | Brewers' spent grain (*Hordeum vulgare*) | VPIP | Alcalase and Flavourzyme | 54,69 | 4 | 493,33 | 0.68 | 100,00% | | VP | VPIP | | | | 3,78 | 0 | Poor | Cermeño et al. (2019) |
| Animal | Camel whey protein (*Camelus dromedarius*) | VPF | Trypsin | 55,10 | 3 | 520,50 | 0.95 | 66,67% | | VP | VPF | PF | | | 3,67 | 0 | Poor | (Nongonierma, Cadamuro, le Gouic et al., 2019) |
| Plant | Wheat gluten hydrolysate (*Triticum aestivum*) | LPQ | Ginger protease (Zingibain) | 56,70 | 3 | 540,50 | 0.78 | 66,67% | | LP | LPQ | | | | 3,70 | 0 | Poor | Taga, Hayashida, Kusubata, Ogawa-Goto, and Hattori (2017) |
| Animal | Whey protein isolate | LKPTPEGDLEIL | Thermoase PC10F | 57,00 | 12 | 293,91 | 0.49 | 58,33% | | | LKPT | | | | 3,69 | −2 | Good | Lacroix et al. (2016) |
| Animal | Boarfish (*Capros aper*) | APLER | Alcalase and Flavourzyme | 63,67 | 5 | 334,25 | 0.24 | 60,00% | AP | AP | APLE | | | | 6,93 | 0 | Good | Harnedy-Rothwell et al. (2020) |
| Animal | Casein-derived peptides | FLQP | *In silico* (prolyl oligopeptidase) | 65,30 | 4 | 450,33 | 0.79 | 50,00% | | | FLQP | | | | 3,57 | 0 | Poor | (Nongonierma & FitzGerald, 2013c) |
| Animal | Tilapia skin (*Oreochromis niloticus*) | IPGDPGPPGPPGP | Flavourzyme | 65,40 | 13 | 372,17 | 0.45 | 92,31% | | IP | IPGD | | | | 0,88 | −1 | Good | (Wang et al., 2015) |
| Animal | Boarfish (*Capros aper*) | IPGA | Alcalase and Flavourzyme | 66,37 | 4 | 364,67 | 1 | 100,00% | | IP | IPGA | | | | 3,63 | 0 | Poor | Harnedy-Rothwell et al. (2020) |
| Animal | Sheep skin (*Ovis aries*) | GPAGPOGFPG | Alcalase, Neutrase & Flavourzyme | 67,12 | 10 | n. d. | 0.86 | 80,00% | | GP | GPAG | | | | 3,62 | 0 | Poor | (Wang et al., 2021) |
| Animal | Boarfish (*Capros aper*) | GPSL | Alcalase and Flavourzyme | 68,13 | 4 | 329,00 | 0.99 | 75,00% | | GP | GPSL | | | | 3,63 | 0 | Poor | Harnedy-Rothwell et al. (2020) |
| Animal | Mare whey protein (*Equus caballus*) | TQMVDEEIMEKFR | Papain | 69,84 | 13 | 215,67 | 0.51 | 30,77% | | | | FR | | | 4,04 | −2 | Good | Song, Wang, Du, Ji, and Mao (2017) |

$IC_{50}$ (μM): half maximal inhibitory activity, expressed in μM.
PCL: peptide chain length.
A in Nt: presence of alanine in the last two positions of the N-terminus.
P in Nt: presence of proline in the last two positions of the N-terminus.
P in Nt*: presence of proline in the last four positions of the N-terminus.
F in Ct: presence of phenylalanine in the last two positions of the C-terminus.
W in Ct: presence of tryptophan in the last two positions of the C-terminus.
Y in Ct: presence of tyrosine in the last two positions of the C-terminus.
pI: isoelectric point determined by Innovagen's peptide calculator PepCalc.
Net charge was determined at pH 7 by Innovagen's peptide calculator PepCalc.
Estimated solubility: solubility is estimated since Innovagen's tool does not take into consideration factors such as peptide concentration.

peptides. To this regard, the sequences VVDLVVFFAAAK, TAELLPR, CGKKFVR and AVPANLVDLNVPALLK were obtained from dark tea protein (Zhao et al., 2020) using centrifuge ultrafiltration through 30 kDa and further purification by high-performance liquid chromatography (HPLC). The sequence VVDLVVFFAAAK displayed the highest potency, with $IC_{50}$ = 33.93 μM, while the other peptides presented low inhibition, $IC_{50}$ above 500 μM. As mentioned above, other studies employed subcritical water to extract antidiabetic peptides from *Spirulina platensis* (Hu et al., 2019), identifying some bioactive sequences which were tested for their α-glucosidase inhibition ($IC_{50}$ from 92.78 to 204.18 μM). Finally, the IPP peptide obtained by ripening Parmigiano-Reggiano Cheese was also found to have inhibitory capacity for α-glucosidase with $IC_{50}$ 764.50 μM (Martini et al., 2021).

Some previous efforts have been put to study the structure-activity relationship of α-glucosidase inhibitory peptides (Ibrahim et al., 2018b; Mojica & de Mejía, 2016), indicating that the amino acid composition of the peptide, mainly residues containing a hydroxyl group on their sidechain at their Nt, and positive net charge were important characteristics. We found a large deviation in the PCL of α-glucosidase inhibitory peptides, ranging from 2 to 16 amino acids. Nevertheless, 80% of the peptides were found to have PCLs of 2-10 residues (Table 2). The three most active peptides have PCLs of 6 (FDPFPK, $IC_{50}$ = 7.93 μM), and 9 (AAAPVAVAK, $IC_{50}$ = 13.70 μM, AIGVGAIER, $IC_{50}$ = 14.73 μM) residues. Meanwhile, the three least active peptides have a length of 5 (LPLLR, $IC_{50}$ = 2000.00 μM), 11 (YINQMPQKSRE, $IC_{50}$ = 1215.42 μM) and 7 residues (STFQQQMW, $IC_{50}$ = 1190.94 μM), which indicates that peptide size is not the main factor affecting its α-glucosidase inhibitory activity.

The mechanisms involved in the activity of α-glucosidase inhibitory peptides are not yet well elucidated, but previous studies with quantitative structure-activity relationship (QSAR) models have revealed that hydrophobic amino acid residues of peptides predominantly interact with residues in the active site of α-glucosidase (Acquah, Stefano, & Udenigwe, 2018). Overall, the presence of hydrophobic amino acids was common in the peptides identified and, although we found a large deviation on their relative content in the bioactive sequence, ranging from 0 (GSR, $IC_{50}$ = 20.4 μM) to 100% of content of hydrophobic amino acids (IPP, $IC_{50}$ = 764.5 μM). Despite this variability, our study concluded that 75% of the peptides analyzed presented 40%–60% of hydrophobic amino acids in their sequences (Table 2).

Furthermore, previous studies have determined that peptides inhibiting α-glucosidase might have diverse molecular features (Ibrahim et al., 2018b; Yu et al., 2011; Zielińska et al., 2020). According to the literature the presence of S, T, and Y residues in the last two positions of the Nt (S in Nt, T in Nt and Y in Nt) and that of P, A and M in the last two positions of the Ct (P in Ct, A in Ct, and M in Ct) affected positively the inhibitory activity of the peptides due to inhibitory peptides binding mostly to the α-glucosidase catalytic domain by hydrogen bonds and electrostatic interactions (González-Montoya, Hernández-Ledesma, Mora-Escobedo, & Martínez-Villaluenga, 2018). Table 2 shows that only a minority of the peptides met any of these conditions, where the most repeated factor was the presence of P in the last two positions of the Ct.

We determined the pI, net charge, and estimated solubility of the peptides, with 22 out of the 39 peptides listed in Table 2 showing good solubility. As for the pI, we found that only 3 of the 39 peptides presented pI in the range of the intestinal pH (7–8.5), which may negatively affect their solubility. Regarding the net charge, 29 of the 39 peptides identified in literature presented a net charge of either 0 or +1, 7 peptides showed a net positive charge different than 1 and only 3 peptides showed a negative net charge. This agrees with previous studies (González-Montoya et al., 2018), which proposed that negatively charged peptides may exhibit limited α-glucosidase inhibitory activity. The most active peptide (FDPFPK) presented PCL = 6 and 33% content of hydrophobic amino acids. After analyzing the structural characteristics previously mentioned, we only found the presence of P in the second to last position at the Ct. This peptide showed good solubility and a pI of

6.39 (net charge of zero at pH 7).

The Lineweaver-Burk double reciprocal plot has also been employed to determine the mechanisms by which bioactive peptides can inhibit α-glucosidase. Ibrahim et al. (Ibrahim, Bester, Neitz, & Gaspar, 2018a) investigated computationally designed bioactive peptides and found that the two most active peptides against α-glucosidase exhibited uncompetitive and noncompetitive inhibition modes. Specifically, the peptide SVPA demonstrated uncompetitive inhibition by binding to the substrate-enzyme complex. On the other hand, the peptide SEPA bound to a portion of the active site, but due to the presence of a valine residue, it was unable to fully interact with the active site, resulting in noncompetitive inhibition. A study based on antidiabetic peptides derived from fermented rice bran (Hu et al., 2023) demonstrated that the most active sequence against α-glucosidase exhibited a noncompetitive inhibition mechanism. It appears that the mechanism involves reversible bonding with the Asp 616 and His 674 residues of the enzyme's active site, although the exact mechanism remains unclear. It is worth noting that the current literature on the kinetics and inhibition mode of α-glucosidase inhibitory peptides remains limited. Further work is needed to elucidate the molecular interactions and binding mechanisms of these sequenced peptides with reported activity.

## 4. DPP-IV inhibitory peptides

In regular metabolism, food intake results in the liberation of insulin secretion hormones known as incretins (GLP-1 and GIP) that would affect numerous target tissues in the body acting as endocrine signal to the pancreas. Pancreatic β-cells increase insulin concentration in the bloodstream, with suitable insulin secretion being a key-factor to maintain physiological blood glucose level. Furthermore α-pancreatic cells would reduce glucagon concentration, avoiding glucose production in the liver. Then, blood glucose concentration is maintained at healthy levels (Rivero Pino et al., 2020). The enzyme DPP-IV would degrade incretins in order to regulate its concentration (Kshirsagar, Aggarwal, Harle, & Deshpande, 2011). Nonetheless, T2DM patients have insufficient insulin level in the bloodstream, and they end up by developing insulin resistance, leading to an increase in glucose blood level inadequate to the organism. According to this physiological background, the inhibition of DPP-IV leads to an increase of the half-life of these incretins, causing insulin secretion to be stimulated and subsequently, the blood glucose level is adequately regulated (Nongonierma & FitzGerald, 2019) (Fig. 1C).

Thus, literature was searched for sequences of DPP-IV inhibitory peptides, by following previously described search criteria and including "dipeptidyl peptidase IV inhibitory peptide" as keyword. The total number of peptide sequences inhibiting DPP-IV was 230 (data not shown). The 40 most active DPP-IV inhibitory peptides were selected based on their higher activity (Table 3), including only those with an $IC_{50}$ value up to 20 times the value of tripeptide IPI, a very well-known DPP-IV inhibitor with $IC_{50}$ = 3.5 μM which was chosen as reference (Nongonierma et al., 2018). The peptide sequences were identified from animal or plant origin, with $IC_{50}$ values varying from 0.51 to 69.84 μM. In contrast to peptides with α-glucosidase inhibitory activity, potential DPP-IV inhibitory peptides from insects have been identified (Rivero-Pino, Guadix, & Guadix, 2021), although their activity has not been yet confirmed by measuring the DPP-IV inhibitory activity of the synthetic peptides. Moreover, plant sources, although gaining increasing interest, have not been studied so far as sources for DPP-IV inhibitory peptides. Thus, plant proteins, together with other sustainable sources such as insect or food by-products, present a great potential as substrate for antidiabetic peptides.

As for the enzymes used, 40% of the peptides selected were obtained by hydrolysis with Alcalase—a serine endopeptidase primarily consisting of subtilisin—alone or in combination with other proteases. Hydrolysis combining Alcalase with Flavourzyme, an enzyme cocktail mainly containing exo-peptidases (Merz et al., 2015), has been shown to be considerably efficient for obtaining DPP-IV inhibitory peptides. Previous authors employed a combination of Alcalase and Flavourzyme to hydrolyze boarfish flesh at pH 7 and 50 °C (Harnedy-Rothwell et al., 2020) and brewers' spent grain at pH 9 and 50 °C (Cermeño et al., 2019). The former identified 10 peptide sequences with $IC_{50}$ values ranging from 3.49 to 68.13 μM, while brewers' spent grain hydrolysis produced 4 peptides with $IC_{50}$ = 38.67–54.69 μM. The active sequence GPAGPOGFPG ($IC_{50}$ = 67.12 μM) was obtained from sheep skin hydrolysis with Alcalase (Wang et al., 2021). Hydrolysis of tilapia skin (Wang et al., 2015) and salmon flesh (Li-Chan, Hunag, Jao, Ho, & Hsu, 2012) with Flavourzyme at pH 7.0, and 50 °C released active sequences with $IC_{50}$ ranging from 41.90 to 65.40 μM (Table 3).

Similarly to α-glucosidase inhibition, digestive enzymes were mostly reported in literature to produce DPP-IV inhibitory peptides. The most active peptides were obtained by hydrolysis of antler velvet from Cervidae employing a mixture of pepsin, trypsin, and chymotrypsin, under physiological conditions (Yu et al., 2017). These peptides displayed high inhibitory potency, with $IC_{50}$ values varying from 0.51 to 1.67 μM. Three active tripeptides (VPV, YPI and VPF) were obtained after simulated digestion of camel whey protein, with $IC_{50}$ of 6.60, 35.0 and 55.10 μM, respectively (Nongonierma et al., 2019).

Regarding the average peptide chain length of the 40 most active DPP-IV inhibitory peptides identified (Table 3), it ranged from 3 to 13 amino acids, with an average value of 6 residues. More precisely, 72% of the peptides presented between 3 and 6 amino acids. The three most active peptides have PCLs of 9 (GPAGPQGPR, $IC_{50}$ = 0.51 μM), 10 (PPGLPGSPGQ, $IC_{50}$ = 0.55 μM) and 7 residues (LPQPPQE, $IC_{50}$ = 0.97 μM) (Yu et al., 2017). Meanwhile, the three least active ones have a length of 13 (TQMVDEEIMEKFR, $IC_{50}$ = 69.84 μM), 4 (GPSL, $IC_{50}$ = 68.13 μM) and 10 residues (GPAGPOGFPG. $IC_{50}$ = 67.12 μM).

Although the mechanisms involved in the activity of DPP-IV inhibitory peptides are not yet well elucidated, recent studies on structure-activity relationship analysis and sequential alignment of inhibitory peptides have demonstrated that the hydrophobicity of the amino acids played an important role in the inhibitory activity. Indeed, inhibitory compounds bind to DPP-IV enzyme through a variety of interactions such as salt bridges, hydrophobic interactions, and hydrogen bonds. The predominant mechanism is the interaction between the charged subpocket S2 of the DPP-IV enzyme and the Nt hydrophobic region of the inhibitory peptides (Acquah et al., 2018; González-Montoya et al., 2018). In this study, the presence of hydrophobic residues within the active sequences was quantified, finding that 75% of the peptides had a hydrophobic content over 80%, and only three of the sequences had a content of less than 50% in hydrophobic amino acids (QLRDIVDK, 37.5%; HPF, 33.3%; TQMVDEEIMEKFR, 30.8%).

Moreover, it has been suggested that the presence of amino acids with aromatic rings could improve the potency of DPP-IV inhibitors by forming hydrophobic interactions with the catalytic domain (Ojeda-Montes et al., 2018). After studying the amino acid content of the peptides, it was found that the most present amino acid within the sequences was P—a hydrophobic amino acid with an aromatic chain—appearing in 38 of the 40 peptides (95%). The presence of other aromatic amino acids in the identified peptides is lower, with only 33% of the studied peptides containing one aromatic amino acid different than P.

Previous works (González-Montoya et al., 2018; Nongonierma & FitzGerald, 2013c) have reported that the position of P in the first, second, third, or fourth Nt position positively affects the inhibitory activity of the peptides. This is consistent among the peptides studied since we found that 80% of them had P in the last two positions (P in Nt), rising to 93% when considering the last 4 positions (P in Nt, 4 positions). The presence of A in the first or second position of the Nt (A in Nt), as well as the presence of aromatic amino acids in the last two positions of the Ct (F in Ct, W in Ct, and Y in Ct), have also been determined to positively affect their inhibitory activity (Hsieh et al., 2016). We searched for these characteristics but did not observe that they were

repeated factors in the identified peptides (Table 3). For instance, 10% of the peptides analyzed presented A in Nt or F in Ct, only 5% presented Y in Ct, and none of the peptides contained W in Ct.

Studies on the pI, solubility, and charge of DPP-IV inhibitory peptides have been carried out with the aim of linking these characteristics with the activity of the peptides, however no clear correlation has been found yet (Kęska, Stadnik, Bąk, & Borowski, 2019; Nongonierma, Mooney, Shields, & FitzGerald, 2014). The peptides reported in this study had very low pI values (Table 3), with only 2 peptides having a pI higher than 7 (GPAGPQGPR, pI = 10.84 and HPF, pI = 7.56). Out of the 40 peptides identified, 13 showed a negative net charge, 29 presented a neutral net charge, and only one of the peptides was positively charged at pH 7. Interestingly and despite the high hydrophobicity of the peptides studied, 14 out of the 40 identified peptides showed good solubility. The most active peptide, GPAGPQGPR, was obtained by simulated gastric-pancreatic digestion of deer antler velvet with an $IC_{50}$ of 0.51 μM. This peptide had a PCL of 9, 78% of hydrophobic amino acids and presented P in the second to last position of the Nt (Yu et al., 2017). Table 4 highlights the main findings on the structure-activity relationship for antidiabetic peptides.

Most of the research dedicated to elucidating the inhibition mechanisms of the presented enzymes has focused on DPP-IV, revealing two clearly different mechanisms depending on peptide size. Peptides smaller than 1 kDa can directly interact with the active site of DPP-IV, resulting in competitive inhibition (Nongonierma & FitzGerald, 2013b; You et al., 2022). This occurs because small peptides can easily access the enzyme's active site to bind. For example, tripeptides like IPR and VPW from *Chlorella vulgaris* (Zhu et al., 2017) have been shown to form hydrogen bonds with residues in the catalytic center of the enzyme and establish van der Waals interactions with both sockets (S1 and S2). Specifically, the presence of W at the N-terminus has been associated with increased interaction with the S1 socket. Another study on dipeptides derived from milk proteins (Nongonierma & FitzGerald, 2013a) also demonstrated a competitive inhibition mechanism, with the exception of the dipeptide WV, which acted as a non-competitive inhibitor.

On the other hand, larger peptides can inhibit DPP-IV by interacting with its dimerization sites, leading to more complex mechanisms such as non-competitive or anti-competitive inhibition. This aligns with the findings of a study on 13-long peptides derived from goat milk casein, which demonstrated uncompetitive inhibition (Zhang, Chen, Ma, & Chen, 2015). However, the mechanisms behind the interaction of these larger peptides are still not fully understood (Nongonierma & FitzGerald, 2019).

## 5. Activity prediction *in silico* for antidiabetic peptides

Lately, *in silico* tools have gained attention for the identification and obtaining of bioactive peptides (Barati et al., 2020). To our knowledge, there are no bioinformatic tools available that allow to predict the α-amylase and α-glucosidase inhibitory activities of peptides. Hence, this study aimed to evaluate the correlation between DPP-IV experimental inhibitory activity of the peptide sequences reported in the literature and the theoretical activity predicted by previously developed *in silico* tools as iDPPIV-SCM tool and StackDPPIV. iDPPIV-SCM was the first computational model for predicting and analyzing DPP-IV inhibitory peptides using sequence information. It is based on the Scoring Card Method (SCM), analyzing protein and peptide functions directly from their amino acid sequence without known structural information (Charoenkwan et al., 2020). StackDPPIV has been recently developed and it aims to improve the prediction accuracy of the iDPPIV-SCM by combining five popular machine learning algorithms in conjunction with ten different feature encodings from multiple perspectives in order to generate a pool of various baseline models, as well as using a genetic algorithm based on the self-assessment-report to determine the optimal informative probabilistic features to develop the final meta-predictor (Charoenkwan et al., 2022).

The *in silico* analysis was conducted on the total amount of DPP-IV inhibitory sequences identified (i.e., 230 sequences). Table 3 shows the *in silico* prediction of the inhibition activity for the 40 DPP-IV peptides selected according to the criteria (i.e., $IC_{50}$ up to 20 times higher than the reference value for the tripeptide IPI). The iDPPIV-SCM tool expresses their prediction as a score value, where values over 294 indicate possible DPP-IV inhibitory peptides (Charoenkwan et al., 2020). The StackDPPIV tool expresses the prediction as the probability of inhibition, assigning values 0–1 where only peptides over 0.5 are predicted to inhibit the DPP-IV (Charoenkwan et al., 2022).

The iDPPIV-SCM tool reported that 154 out of the 230 peptides were possible inhibitors of the DPP-IV, while 11 peptides were not detected (i.e., peptides with only one amino acid or peptides with modified amino acids). The peptides with highest activity according to the calculated score were PP (score = 960.00), VPW (score = 596.50) and ER (score = 360.00) with reported $IC_{50}$ values of 4343.48 μM (Neves et al., 2017), 174.78 μM (Xiang et al., 2021) and 4480.00 μM (Lafarga, Aluko, Rai, O'Connor, & Hayes, 2016), respectively. Unexpectedly, their reported *in vitro* inhibitory capacities were significantly poorer, compared to their *in silico* scores. This discrepancy was also observed for the peptides with the highest *in vitro* activity reported in the literature review, GPAGPQGPR, PPGLPGSPGQ and LPQPPQE, with $IC_{50}$ values of 0.51, 0.55 and 0.97 μM respectively. According to the *in silico* analysis, their score values were 320.88, 342.78 and 439.50, placing them at rank 130, 118 and 46 respectively within the original list of 230 inhibitory sequences. Indeed, no significant relationship ($r^2 = 0.0102$) can be found between the $IC_{50}$ values of the 40 most active peptides reported in the literature and the score values calculated *in silico*. This leads to conclude that, although the DPP-IVi tool is very promising, it still has limitations because it mainly predicts bioactivity only based on the propensity scores of 20 amino acids. It is also worth mentioning that 60% of the peptides used for the DPP-IVi tool database were composed of 5 or less amino acids, which differs from the PCL range of DPP-IV inhibitory peptides reported in the literature.

Regarding the data obtained with the StackDPPIV, 164 out of the 230

**Table 4**
Main relationships between structural features and activity for antidiabetic peptides.

| Enzyme inhibited | PCL | AA in Nt | AA in Ct | Hydrophobicity | Aromatic AAs | Solubility | Net charge |
|---|---|---|---|---|---|---|---|
| α-amylase | ~70% between 3 and 10 AAs | >50% present L or P in first 2 positions | 10% present F in last 2 positions | >44% hydrophobic AAs | – | >65% low | ~60% positive |
| α -glucosidase | ~80% between 2 and 10 AAs | ~35% present S, T or Y in first 2 positions | 30% present P or A in last 2 positions | 75% contain ~50% hydrophobic AAs | – | >55% good | ~75% neutral or positive |
| DPP-IV | >70% between 3 and 6 AAs | >90% present P in first 4 positions | – | 75% contain >80% hydrophobic AAs | 95% contain P | 65% low | >70% neutral |

PCL: peptide chain length.
AA in Nt: amino acids in N-terminal.
AA in Ct: amino acids in C-terminal.
Net charge at pH 7.

peptides identified in literature were possible inhibitors of DPP-IV, while 6 peptides were not detected (i.e., peptides with only one amino acid or peptides with modified amino acids). From these 164 peptides, 24 of them were assigned with the highest probability value, while their reported $IC_{50}$ values ranged from 5.61 μM (Harnedy-Rothwell et al., 2020) to 9690 μM (Gallego, Aristoy, & Toldrá, 2014). Considering the 40 selected peptides (Table 3), 4 of them were assigned the maximum probability of 1: IPV, VPV, LPIA and IPGA with reported $IC_{50}$ values of 5.61 μM (Harnedy-Rothwell et al., 2020), 6.6 μM (Nongonierma et al., 2019), 45.07 μM (Cermeño et al., 2019) and 66.37 μM (Harnedy-Rothwell et al., 2020), respectively. Up to 20% of the peptides were predicted to not be inhibitors of the enzyme DPP-IV. The peptide with the least probability estimated was ASGLCPEEAVPRR with 0.06 value and an $IC_{50}$ of 2.2 μM (Thakur et al., 2021). As for iDPPIV-SCM, there was no correlation between the probability value calculated by the StackDPPIV and the *in vitro* activity reported in literature ($r^2 = 0.0041$). Moreover, the correlation between the predicted results obtained from both tools was also compared but a poor correlation was found ($r^2 = 0.270$). This lack of correlation between the experimental data and the predictors shows that *in vitro* analyses by conventional enzymatic means are still necessary to assay the activity of these peptides. However, both the iDPPIV-SCM and the StackDPPIV could be used as a preliminary guidance tool to estimate in a qualitative way (yes/no) whether the peptides could express inhibitory activities.

## 6. Evidence of antidiabetic activity of peptides in cell models and *in vivo* studies

Despite the importance of *in vivo* studies investigating the physiological effects of antidiabetic peptides, most of the recent studies focus on *in vitro* or *in silico* evaluations. *In vivo* evaluation is specially needed considering that the bioavailability of the peptides can be greatly affected both by hydrolysis of peptidases in the stomach and by intestinal brush border membrane enzymes (Liu, Cheng, & Wu, 2019).

Some of the literature found used cell models to analyze the inhibition of DPP-IV, and to a lesser extent of α-amylase and α-glucosidase, in cells. Harnedy-Rothwell et al. studied the DPP-IV inhibitory activity of boarfish peptides in Caco-2 cells (Harnedy-Rothwell et al., 2020). This cell model simulates intestinal mucosal conditions to analyze the ability of the peptides to pass through human intestinal cell membranes and resist degradation by brush border enzymes. In general, they found that all peptides were able to exert antidiabetic activity, albeit to a lesser extent. Similar results were obtained with peptides obtained from sorghum bicolor seed (Majid, Lakshmikanth, Lokanath, & Poornima Priyadarshini, 2022) and silver carp swim bladder hydrolysates (Hong et al., 2020). No significant difference was found in the DPP-iv inhibitory activity of sheep skin peptides both *in vitro* and in the cell model (Wang et al., 2021).

Another frequently used model is HepG2 cell, which show deficient glycogen synthesis and failure to suppress glucose production. This model was used to measure the antidiabetic activity of *Spirulina platensis* peptides, which had previously showed α-amylase, α-glucosidase, and DPP-IV inhibition (Hu et al., 2019). The peptides significantly increased the glycogen content and glucose metabolism enzymes activities, lowering blood sugar and improving insulin resistance. Zhang et al. also used HepG2 cells to demonstrate the antidiabetic activity of common carp roe peptides (Zhang et al., 2020).

Regarding *in vivo* assays, very little information was found in the literature. Rats and mice were the most commonly used animals, since they are easy to handle and cost-effective. High-fat diet/streptozotocin-treated (HFD/STZ) rats were used to determine the *in vivo* activity of tilapia skin gelatin peptides, which had inhibited DPP-IV activity *in vitro* (Wang et al., 2015). This study demonstrated that fish skin gelatin hydrolysates had dual actions of DPP-IV inhibition and GLP-1 secretion enhancement, improving glycemic control in the rats after only 30 days. Other studies have also demonstrated the *in vivo* ability of fish-derived peptides to regulate the glycemic index. The glucose-lowering and insulin releasing properties of blue whiting muscle protein hydrolysates was studied using the Oral Glucose Tolerance Test (OGTT) in NIH Swiss mice (Harnedy et al., 2018). This test consists of administering a preload of the bioactive peptides followed by a glucose load and measuring the blood glucose levels at different times. They found that the hydrolysates mediated insulin and glucagon-like peptide-1 (GLP-1) release, increasing its secretion. Furthermore, they produced glucose-lowering effects both acutely (at 90–120 min after glucose load) and persistently (at 4h after glucose load). The antidiabetic effect of sturgeon collagen hydrolysates by *in vitro* analysis of α-glucosidase and DPP-IV and by OGTT in Institute of Cancer Research (ICR) mice was analyzed, obtaining positive results (Sasaoka et al., 2021).

The most frequent method of administration was via oral, an easier to perform and safer method compared to intraperitoneal administration (Nong & Hsu., 2021). Some studies tested the OGTT on ICR mice fed with Yam tuber peptides (Lin, Han, Lin, & Hou, 2016) and casein-derived peptides (Zheng et al., 2019). They found that postprandial blood glucose levels were reduced. The *in vitro* activity of brewer's spent grain hydrolysates was studied *in vivo* (Cermeño et al., 2019), where wistar rats were supplemented via oral with encapsulated brewer's spent grain hydrolysates and found that the activity of α-amylase, α-glucosidase and DPP-IV was reduced, and serum glucose levels decreased (Garzón et al., 2022). Only one study used the via intraperitoneal to treat the tested NIH Swiss mice. They went on to investigate *Palmaria palmata* peptides which had previously shown DPP-IV inhibitory activity *in vitro* (Harnedy, O'Keeffe, & FitzGerald, 2015), testing the effect of injecting glucose alone or in combination with the bioactive peptides. They found that these peptides were able to act as glucose-dependent insulinotropic polypeptide (GIP) secretagogues and could therefore be used in combination with drugs to aid in the prevention and management of diabetes (Harnedy-Rothwell et al., 2021). Finally, the effect of oral and intraperitoneal supplementation was studied, using soybean-derived peptides in alloxan-induced diabetes Kunming mice, finding stronger results via oral (Jiang, Yan, He, & Ma, 2018).

Studies showed promising results for the management of T2DM with antidiabetic peptides. However, literature is still lacking, and further work should be carried out testing *in vivo* activity.

## 7. Potential degradation of active peptides during digestion

One of the most effective approaches to ingest bioactive peptides is by using them as bioactive ingredients in functional foods (Tadesse & Emire, 2020). Nonetheless, there is a risk that these peptides are degraded by the effect of gastrointestinal proteases and serum peptidases during the digestion process (Sun, Acquah, Aluko, & Udenigwe, 2020). Although not all peptides are equally susceptible to this enzymatic degradation, those that are altered can reduce their activity or could enhance it in case the new released peptides are more active compared to the parent peptide. In this regard, the use of *in silico* tools is useful to simulate digestion and predict which of the identified peptides can be degraded and at which specific point they are attacked by the native enzymes present. PeptideCutter (Maillet, 2020) was used to perform an *in silico* gastrointestinal digestion of the peptides identified by entering the peptide sequences and predicting the potential sites cleaved by pepsin (pH: 1.3), chymotrypsin (low and high specificity) and trypsin (Barati et al., 2020). This tool allowed us to predict the potential new species produced after digestive degradation.

It was found that longer peptides are more susceptible to being digested, as they have more possible cleavage sites. This is confirmed by data shown in Tables 1-3S (supplementary material), where 15 of the 18 α-amylase inhibitory peptides (average PCL = 10) undergo at least one modification, 27 of the 39 α-glucosidase inhibitory peptides (average PLC = 8) are modified, and only 19 of the 40 DPP-IV inhibitory peptides (average PLC = 6) are modified. The peptides with α-amylase inhibitory

activity can be highly hydrolyzed by digestion, finding that the most active sequence (FFRSKLLSDGAAAAKGALLPQYW) can be attacked by the different enzymes at up to 11 different cleavage sites. For α-glucosidase and DPP-IV inhibitory peptides, this rate of degradation is much lower, where the most inhibitory sequences of each enzyme (FDPFPK and GPAGPQGPR) have only one cleavage site where they can be cleaved.

To study the potential effect of peptide degradation on their activity iDPPIV-SCM was used to predict the bioactivity of the new released peptides. Although iDPPIV-SCM computational tool did not present a good correlation with the inhibitory activity obtained *in vitro*, it was selected as qualitative predictor of inhibitory activity of the released peptides (score>294.0 for active peptides). iDPPIV-SCM was selected over StackDPPIV tool, due to the much extended use of the former in the literature. From the 40 DPP-IV inhibitory peptides digested, 47 peptide fractions were obtained, 30 of which would maintain their activity according to the computational tool, and 17 would lose their inhibitory activity or were not able to be detected (Table 3S).

In any case, it is worth noting that the results obtained are indicative, considering the wide limitations shown by both computational predictors and the PeptideCutter tool. Although PeptideCutter tool allows us to estimate the alteration that peptides would undergo during gastrointestinal digestion, it should be taken into account that hydrolysis could not be performed at 100%, and not all the bonds susceptible to attack would actually be broken. Hence, it would be necessary to study experimentally the real effect of digestion, since this *in silico* analysis can only serve as an approximation of the digestive effect, as it does not consider other factors affecting peptide stability, such as pH. For instance, the acid pH found in the stomach can modify the structure, charge, and interaction capacity of the peptides, thus limiting their activity (Marcolini et al., 2015). Thus, it would be advisable to carry out *in vitro* studies using the INFOGEST method (Brodkorb et al., 2019) to investigate how gastrointestinal digestion can affect the degradation and activity of antidiabetic peptides. To our knowledge, only one work has been reported regarding the effect of *in vitro* gastrointestinal digestion of synthetic antidiabetic peptides (Rendón-Rosales et al., 2022), which resulted in degradation of most of the peptides after digestion and increased inhibition of DPP-IV for 7 out of the 12 peptides. This lack of literature highlights the need to focus research on the effect of gastric digestion on the bioactivity of antidiabetic peptides.

## 8. Conclusions

*In vitro* analyses to determine the inhibitory activity of α-amylase, α-glucosidase and DPP-IV by peptides serve as initial assessment to establish if these food-derived molecules obtained by enzymatic hydrolysis can have potential bioactivity to prevent or pre-treat diabetes. Peptides identified from different sources, with an experimentally determined $IC_{50}$ to inhibit α-amylase, α-glucosidase, and DPP-IV were reviewed. A critical assessment of the data suggests that the methodology employed among authors is not consistent in some cases (e.g., for α-amylase and α-glucosidase), as the $IC_{50}$ for the positive control (acarbose) varies among authors. This hinders the complete comparison of results. Nevertheless, for DPP-IV inhibitory peptides, the methodology is highly identical among authors. Although *in silico* tools are gaining attention to identify antidiabetic peptides, no correlation was found between the experimental DPP-IV inhibitory activity of the peptides and the one predicted by the iDPPIV-SCM and StackDPPIV tools. Thus, further development of the bioinformatic tools is required. Although the number of α-amylase and α-glucosidase inhibitory peptides identified so far is low, the relationship between the structural features of the reported peptides and their activity has been discussed. For DPP-IV inhibitory peptides, the presence of P at the Nt is found to be a highly conserved feature. It was found that α-amylase inhibitory peptides have the longest PCLs (11 amino acids average), whereas DPP-IV inhibitors are the shortest (6 amino acids average), which would justify that their sequences are much less degraded during gastrointestinal digestion *in silico*.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102954.

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