Research Article

Computational Screening and Molecular Docking Analysis of Bioactive Peptides from Spent Coffee Grounds as Potential α-Glucosidase and α-Amylase Inhibitors for Antidiabetic Therapy

Vanarat Phakeenuya
Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
Microbial Informatics and Industrial Product of Microbe Research Center, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

Theerawut Phusantisampan*
Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
Biorefinery and Process Automation Engineering Center, Department of Chemical and Process Engineering, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

Malinee Sriariyanun
Biorefinery and Process Automation Engineering Center, Department of Chemical and Process Engineering, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

* Corresponding author. E-mail: phusantisampan.t@gmail.com DOI: 10.14416/j.asep.2025.09.002
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Abstract
A metabolic disease, type 2 diabetes mellitus (T2DM), is marked by chronic hyperglycemia due to insulin resistance or impaired secretion. Inhibiting carbohydrate-digesting enzymes, particularly α-glucosidase and α-amylase, is a therapeutic approach to regulate blood glucose levels. Protein components of spent coffee grounds (SCG), a byproduct of coffee production, can be hydrolyzed to produce bioactive peptides with potential health benefits. In this study, the 11S storage protein of SCG was simulated with alcalase digestion for peptide synthesis. These peptides were computationally screened for antidiabetic potential using bioactivity prediction tools and were evaluated for bioactivity, toxicity, bitterness, blood stability, and protein-binding potential. Most were predicted to be non-toxic, non-bitter, and had favorable blood half-lives (>830 s), suggesting therapeutic viability. Molecular docking was performed against α-glucosidase and α-amylase to assess binding affinity. The amino acids TRP406, ARG526, and ASP542 of α-glucosidase were strongly bound by the peptides GRPQPRL and RRF, which had binding strengths of -8.5 and -8.3 kcal/mol, respectively. Meanwhile, ASP197, GLU233, ASP300, HIS299, and HIS305 were key components of α-amylase's binding with APHW (-8.7 kcal/mol). The presence of aromatic and polar residues contributed to binding strength and stability in enzyme active sites. These results indicate that SCG-derived peptides have promising inhibitory activity against α-glucosidase and α-amylase and may serve as natural, safe, and stable candidates for developing functional antidiabetic therapies.

Keywords: Alcalase digestion, Bioactive peptides, Biorefinery, Metabolic disease, Molecular docking, Spent coffee grounds

1 Introduction

Diabetes is not stable in blood sugar levels, sensitivity, and limited insulin secretion; it is a chronic metabolic disease with a high prevalence worldwide, and annually, the number of patients is increasing [1]. Over half a billion people worldwide are anticipated to have diabetes mellitus in 2021, and an estimated prediction is that it will increase to nearly 800 million adults by 2045 [2]. All occurrences of diabetes, 90 to 95 percent are type II diabetes mellitus (T2DM), the most common kind of the condition, which is a usual feature of postprandial or persistent hyperglycemia. Synthetic drugs for diabetes are acknowledged for their efficacy in regulating blood glucose levels by enhancing insulin production, augmenting insulin sensitivity, or inhibiting glucose absorption pathways [3]. Many synthetic medicines, however, have been linked to undesirable side effects, such as weight gain, gastrointestinal distress, and a higher risk of hypoglycemia [4]. Some of the newer antidiabetic medicines are quite expensive and require constant attention, which can be a big financial burden in the long run [5]. This has led to the discovery of active compounds from food that can aid in glycemic regulation, potentially providing safer and more cost-effective alternatives for diabetes management [1]. An α-amylase (EC 3.2.1.1) starts the process of breaking down starch and produces maltose, maltotriose, and limit dextrins [6], [7]. However, the digestive enzyme α-glucosidase (EC 3.2.1.20) primarily hydrolyzes disaccharides into glucose, which mostly enters the bloodstream and raises the glycemic index [8]. An effective and straightforward method of managing T2DM is the use of inhibitors to regulate α-amylase and α-glucosidase activity to delay the small intestine's digestion of carbs and help maintain blood glucose homeostasis. Acarbose, miglitol and voglibose are examples of carbohydrate-hydrolyzing enzyme inhibitors that are utilized in clinical treatment. However, liver disorder, diarrhea and flatulence are some of the adverse consequences of these inhibitors [9]. The α-glucosidase and α-amylase inhibitors are naturally occurring substances that are produced from dietary proteins, namely bioactive peptides. Their long-term use, high safety, and strong biocompatibility have made them a popular research topic. As a result, numerous studies using *in vitro*, *in vivo* and *in silico* methods have examined the bioactive potential of plant and animal proteins [10], [11].

Producing protein hydrolysates from food waste shows promise for increasing value and reducing the financial losses caused by the issue of food waste overload [12]. Food waste is converted into bioactive peptides that show activity against antibacterial, antihypertensive, anti-inflammatory, and anti-hyperglycemic qualities [13]. Recent studies have reported several antidiabetic peptides that inhibit key enzymes involved in carbohydrate breakdown. α-glucosidase is inhibited by peptides isolated from scallops [14], and oat and buckwheat peptides show similar activities [13]. α-amylase activity has been demonstrated to be inhibited by peptides derived from cumin seeds [15]. The α-glucosidase and α-amylase enzymes have been found to be inhibited by peptides obtained from black beans [15], quinoa [16], and lupin [17]. To create α-glucosidase and α-amylase inhibitory peptides, a suitable food-derived protein must be chosen. A key tool in the conversion of protein-rich waste into bioactive peptides is a commercial alcalase, an available serine endopeptidase that was isolated from *Bacillus licheniformis* [18]. The food industry makes extensive use of alcalase because of its high catalytic efficiency, broad substrate specificity, and capacity to operate throughout a broad pH and temperature range [19]. It has been successfully applied in hydrolyzing various protein sources such as lupin [17], *Lemna minor* [20] and whiteleg shrimp [21]. Its robust performance and food-grade status make alcalase an ideal candidate for the enzymatic treatment of agro-industrial byproducts [18].

Coffee beans that have been ground and roasted after losing part of their water-soluble components are known as spent coffee grounds (SCG), whose disposal is a significant issue from an environmental perspective. Furthermore, current estimates indicate that the world produces 6 million tons of SCG annually [22]. Consequently, the need for appropriate SCG disposal techniques emerged. The main components of SCG are carbohydrate, lipid, protein and phenolic compounds. As a result, SCG was used to extract value-added products that have a wide range of uses, including energy, chemicals, and materials [22]. However, bioactive peptides of SCG were reported as an antihypertensive and angiotensin I-converting enzyme (ACE) inhibitor [23]. Furthermore, protein hydrolysates obtained from *Bacillus clausii*-

fermented SCG have been predicted to possess potential bioactivity through *in silico* analysis [24]. Based on these findings, Jasus *et al*., chose peptides with high predicted bioactivity scores from the previous study, synthesized them, and subsequently evaluated their inhibitory effects on α-glucosidase. The results showed that α-glucosidase activity was greatly reduced [25]. Therefore, there remains limited information regarding the identification of bioactive peptides with α-glucosidase and α-amylase inhibitory activities derived from SCG hydrolyzed using commercial enzymes. Investigating the inhibitory potential of these two enzymes to promote environmental sustainability and increase SCG's value is therefore important.

Recently, *in silico* techniques have revolutionized the early-stage discovery of bioactive peptides by allowing researchers to simulate enzymatic hydrolysis, predict peptide sequences, and model their interactions with biological targets using molecular docking. These computational tools save time and cost with experimental screening and have proven effective in identifying peptides with specific enzyme-inhibitory properties [26]. This study aimed to investigate whether SCG bioactive peptides can block α-glucosidase and α-amylase using bioinformatics tools. The process of creating and testing bioactive peptides from SCG using computer simulations includes breaking them down with alcalase, identifying the peptides, checking their properties, and conducting a molecular docking study. This approach contributes to the development of functional ingredients for metabolic health and aligns with global sustainability goals through valorizing food processing waste into value-added products.

2 Materials and Methods

2.1 *In silico simulated Alcalase digestion of the coffee protein alignments*

The 11S storage protein of the *Arabica coffee* sequence (GenBank: CAA76573.1) was downloaded from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). This protein sequence was hydrolyzed by the enzyme alcalase using NovoPro's Protease Digestion Tool (https://www.novoprolabs.com/tools/peptide-protease-digestion). Using bioinformatics methods, hydrolyzed peptide sequences have been identified as bioactive peptides for subsequent investigation.

2.2 *Identification of bioactive peptide candidates implicated in α-glucosidase and α-amylase inhibition by bioinformatics tools*

The Peptide Ranker service (http://distilldeep.ucd.ie/PeptideRanker/) was used to analyze the bioactivity of peptide sequences. Subsequently, the PepSite2 server (https://pepsite2.russelllab.org/) was used to analyze the α-glucosidase and α-amylase inhibitory activities of the peptide sequences that had a ranker score greater than 0.5. The most effective peptide candidates and their possible binding sites to inhibit α-glucosidase and α-amylase were chosen from the peptide sequences, which showed a significant level below 5% (p-value < 0.05).

2.3 *Toxicity, bitterness, and stability of the peptide candidate assessments*

The peptide candidates were analyzed for cytotoxicity by ToxinPred (http://crdd.osdd.net/raghava/toxinpred/). BERT4Bitter (http://pmlab.pythonanywhere.com/ BERT4Bitter) was used as the bitterness screening tool. PLifePred (https://webs.iiitd.edu.in/raghava/plifepred/) was used to analyze its stability in blood.

2.4 *Molecular docking studies and validation*

The RCSB Protein Data Bank provided the three-dimensional structures of α-glucosidase (PDB ID: 2QMJ) and α-amylase (PDB ID: 2QV4). Three-dimensional peptide structures were created and saved in PDB format using the PEP-FOLD3 (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) internet server [27]. The ligand molecular structure of acarbose (Compound CID: 41774) was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The experimental binding poses and energies [28] were predicted by AutoDock Vina 4.2.6 (The Scripps Research Institute, La Jolla, San Diego, CA, USA) [29], which was used to determine the binding modes of each ligand bound to the substrate-binding sites of α-glucosidase and α-amylase. Gasteiger partial charges were assigned, and polar hydrogens were introduced [30]. Table 1 displays the search grid for each receptor's key site. To improve docking accuracy, the exhaustiveness value was set to 300. After docking analysis, the protein-ligand interactions were

visualized by BIOVIA Discovery Studio Visualizer v20.1.0.0 software (Accelrys, San Diego, CA, USA).

The docking parameters were validated re-docking method. The co-crystallized ligand was re-docked into the active site to accomplish this [31]. The goal was to ensure that the ligand correctly binds to the active site cleft with the least variation from the native co-crystallized complex. Using the identical grid parameters and methodology for each enzyme. Each initial co-crystallized complex's native ligand was removed and re-docked into the active site using the same grid parameters and procedure for both enzymes (Table 1). The native co-crystallized structure was used as the reference structure for the positioning of the re-docked complex, and then the root mean square deviation (RMSD) between the docked and co-crystallized positions for these two enzymes was calculated.

Table 1: Lists of each receptor's docking box dimensions and center coordinates.

Receptor	Center	Dimension Size
α-glucosidase (2QMJ)	X: -23.755 Y: -5.190 Z: -11.162	X: 40 Y: 50 Z: 42
α-amylase (2QV4)	X: 10.749 Y: 48.629 Z: 21.111	X: 56 Y: 66 Z: 60

3 Results and Discussion

3.1 *Simulated alcalase digestion*

One of the key proteins found in *Coffea arabica* seeds is the 11S storage globulin, which weighs about 55 kDa [32]. The 11S storage globulin sequence contains 492 amino acids, which were acquired from NCBI (GenBank: CAA76573.1). We used NovoPro's Protease Digestion Tool to simulate how alcalase breaks down the 11S storage globulin to predict the sequences of bioactive peptides. Figure 1 exhibited cleavage mapping sites of 11S storage globulin with alcalase. The result revealed that 43 cleavage sites produced 144 protein fragments (including single amino acids). The results showed that alcalase cleaved globulin S11 in several places, especially after hydrophobic amino acids (Figure 1). This cleaving pattern is similar to the Adamson and Reynolds (1996) report, which presented that alcalase tended to break peptide bonds when the amino acids GLU, MET, LEU, TYR, LYS, and GLN appeared. Alcalase can thus yield peptides with broad hydrophobic properties [33]. The size of peptides was divided into 3 groups: small peptides (2-10 amino acid residues) weighing 200–1200 daltons [11], moderate peptides with 11–30 amino acid residues weighing 1,300–3,500 daltons [34], and large peptides (31 or more than 50 amino acid residues) weighing 3,600 or more than 6,000 daltons [35]. Furthermore, the results exhibited various amino acids, small-sized peptides, and some moderate-sized peptides when hydrolyzed with alcalase (Figure 1). These results agree with other studies that revealed a broad range of amino acids, which detect the protein breakdown caused by alcalase, and it tends to produce a hydrolysate with many small peptides [33]. These cleavage events modify both the size and amino acid composition of the resulting hydrolysates, producing short-chain peptides with exposed hydrophobic side chains. These hydrophobic residues enhance binding affinity for α-glucosidase and α-amylase [17], [36], but they may also impart a bitter taste, potentially reducing sensory appeal [37].

M – AHSHM – I – SL – SL – Y – V – L – L – F – L – GCL – AQL – GRPQPRL– RGKTQCDI – QKL – NAQEPSF – RF – PSEAGL – TEF – W – DSNNPEF – GCAGV – EF – ERNTV – QPKGL – RL – PHY – SNV – PKF – V – Y – V – V– EGTGV – QGTV – IPGCAETF – ESQGESF – W – GGQEQPGKGQEGQEQGSKGGQEGRRQRF – PDRHQKL – RRF – QKGDV – L – I – L – L – PGF – TQW – TY – NDGDV – PL – V – TV – AL – L – DV – ANEANQL – DL – QSRKF – F – L – AGNPQQGGGKEGHQGQQQQHRNI – F – SGF – DDQL – L – ADAF – NV – DL – KI – I – QKL – KGPKDQRGSTV – RAEKL – QL – F – L – PEY – SEQV – QQPQQQQEQQQHGV – GRGW – RSNGL – EETL – CTV – KL – SENI – GL – PQEADV – F – NPRAGRI – TTV – NSQKI – PI – L – SSL – QL– SAERGF – L – Y – SNAI – F – APHW – NI – NAHNAL – Y – V – I – RGNARI – QV – V – DHKGNKV – F – DDEV – KQGQL – I – I – V – PQY – F – AV – I – KKAGNQGF – EY – V – AF – KTNDNAM – I – NPL – V – GRL– SAF – RAI – PEEV – L – RSSF – QI – SSEEAEEL – KY – GRQERL – L – L– SEQSQQGKKRSCL – S

Figure 1: *In silico* simulated digestion mapping of coffee 11S globulin with the alcalase enzyme. Cleavage sites of this enzyme are indicated by dashes between amino acid residues.

3.2 *In silico prediction of 11S storage globulin bioactive peptides*

PeptideRanker and PepSite2 servers were used to predict the α-glucosidase and α-amylase inhibitory activities of 144 protein fragments (consisting of 49 amino acid residues and 95 peptides) that were derived by simulated alcalase digestion (Figure 1). The popular tool of peptide bioactivity prediction is Peptide Ranker, which performs with machine learning algorithms that have been trained on several peptide databases to determine the biological activity probability of peptide sequences [38]. Peptide ranker

score values closer to 1 indicate a higher probability of bioactivity. A total of 95 peptides were analyzed, and 59 of them had a Peptide ranker score above the cutoff of 0.5, which suggested these peptides might be active biologically. The results of Peptide Ranker analysis exhibited that several peptides derived from the alcalase hydrolysis of 11S storage globulin had the potential to be bioactive. This is consistent with other studies that exhibited seed storage proteins, particularly 11S globulins, served as rich precursors for bioactive peptides with antihypertensive, antioxidant, and antimicrobial activities [11], [34]. Fifty-nine of the selected peptides with Peptide Ranker scores above 0.5 were further analyzed using PepSite2, a structure-based peptide-protein interaction prediction tool, which is particularly used for identifying interaction hotspots without the need for full docking simulations. It is a valuable tool for peptide-based drug discovery and functional peptide research. Potential peptides that are involved in protein or enzyme binding sites were chosen at a significant level below 5% (p-value <0.05) [39]. According to the PepSite2 results, 24 peptides exhibited a p-value score less than 0.05, suggesting that they can bind to the α-glucosidase active sites (Table 2). However, peptide EF, ADAF and GRGW exhibited p-value scores exceeding 0.05, indicating that they did not interact with the α-amylase active sites (Table 2). The results indicated that several peptides from coffee 11S globulin have outstanding potential as α-glucosidase and α-amylase inhibitors, which are important therapeutic targets for controlling postprandial hyperglycemia in type 2 diabetes. A total of 24 peptides were further evaluated using *in silico* approach for toxicity (ToxinPred), bitterness (BERT4Bitter), and stability in blood (PLifePred). All peptides were safe candidates for food or nutraceutical applications because they were predicted to be non-toxic (Table 2). Additionally, the stability of peptides is crucial for evaluating their pharmacokinetics and therapeutic potential. Peptide breakdown in blood affects their effectiveness and availability in the body, showing that they last longer than 800 seconds in tests that simulate blood [40]. All peptides revealed favorable stability in blood (Table 2). Nevertheless, peptides consisting of GRPQPRL, RF, EF, RRF, PGF, ADAF, GRGW, AF and GRL were predicted as bitter. Hydrophobic amino acids like phenylalanine (F), leucine (L), valine (V), isoleucine (I), tyrosine (Y), tryptophan (W), proline (P), and methionine (M) are the main factors that affect a peptide's bitter taste [37]. Additionally, the C-terminal end of these nine peptides had hydrophobic residues that had a high interaction with the bitter taste receptors. This result was consistent with Kuroda & Miyamura's, which showed that dipeptides and tripeptides containing F, L, or W at the C-terminal end often stimulate bitter taste [41]. The crucial sensory characteristic of peptide bitterness influences functional food and nutraceutical formulations. To make bioactive peptides that are both useful and appetizing, it is essential to understand the relativity of peptide sequence and taste interactions.

3.3 *Molecular interaction of candidate peptides with α-glucosidase and α-amylase*

Table 2 showed that peptide sequences were expected to be able to interfere with α-glucosidase and α-amylase activity (p-value < 0.05). These candidate peptides were small chains of 2–7 amino acids, weighing between 228.31 and 823.06 daltons (Table 2). For additional binding potential analysis using PepSite2, we divided these candidate peptides into five groups according to the peptide's size. Table 3 indicated that all peptides attached to α-glucosidase (PDB ID: 2QMJ) at important amino acid residues, which were already known to be essential for recognizing and processing substrates, including ASP327, ILE364, TRP406, TRP441, ASP443, ARG526, ASP542, PHE575, and HIS600 [42]. According to Table 3, dipeptides, RF, EF, RL, PL, and AF interacted with several key residues, including TRP406, ARG526, and ASP542. The results exhibited that the peptides RF, EF, and AF had similar key amino acid residues, while RL and PL revealed different sets of amino acid residues. Additionally, peptide RF could attach to eight spots on the enzyme, making it the best peptide in this group at blocking the activity of the α-glucosidase enzyme. In contrast, peptide PL showed the lowest binding ability with 4 key amino acid residues. However, tripeptides consisting of PKF, RRF, GRL, and SAF interacted with 8 key amino acid residues of the α-glucosidase enzyme. Tetrapeptide APHW was the most effective at blocking α-glucosidase, attaching to 9 amino acid residues, while the other peptides attached to 7 or 8 amino acid residues (Table 3). Moreover, the longer peptides (penta- and heptapeptides) showed a wider range of interactions by connecting with the active site and nearby residues,

ASP443 and MET444, indicating they might work in different ways to block enzyme activity. In this case, the peptides QSRKF and GRPQPRL exhibited the strongest binding ability for inhibiting α-glucosidase, each containing 10 amino acid residues. These results indicated that the hydrophobic and aromatic residues of peptides were involved in α-glucosidase binding sites (Table 3). This is consistent with earlier research that found these residues are crucial for α-glucosidase inhibition [43]. Additionally, the results of this study were similar to Fadimu's report (2022), which exhibited a large number of peptides that exhibited hydrophobic or aromatic amino acid residues, particularly at the C-terminal end, generated by alcalase [17]. Furthermore, Additionally, the peptides SPRRF, FE, and RR were shown to be the strongest α-glucosidase inhibitors, indicating that the presence of basic and hydrophobic amino acids aided in the inhibition of α-glucosidase [17].

Table 2: *In silico* prediction of molecular weight, toxicity, bitterness and stability of the peptide candidates using computational screening tools.

Sequences	Number of Amino Acids	Mass (Da)	Peptide Ranker Ratio	PepSite2		Toxicity	Bitterness	Stability
						ToxinPred	BERT4Bitter	PLifePred
				α-glucosidase	α-amylase	Score (*)	Score (*)	Half-Life in Blood (s)
GCL	3	291.40	0.913	0.0038220	0.0242800	-0.74 (NT)	0.00 (NB)	834.71
GRPQPRL	7	823.06	0.810	0.0000397	0.0013610	-1.32 (NT)	0.97 (B)	839.71
RF	2	321.39	0.986	0.0006759	0.0044690	-0.80 (NT)	0.83 (B)	834.81
DSNNPEF	7	821.88	0.554	0.0022130	0.0215800	-0.66 (NT)	0.12 (NB)	858.01
EF	2	294.32	0.598	0.0457100	0.1620000	-0.80 (NT)	1.00 (B)	834.81
QPKGL	5	541.72	0.501	0.0001120	0.0002436	-0.83 (NT)	0.16 (NB)	837.81
RL	2	287.38	0.626	0.0047710	0.0457100	-0.80 (NT)	0.01 (NB)	834.81
PHY	3	415.48	0.565	0.0002987	0.0004134	-0.81 (NT)	0.00 (NB)	834.61
PKF	3	390.51	0.894	0.0002977	0.0002709	-0.80(NT)	0.00 (NB)	834.61
PGCAETF	7	723.88	0.637	0.0055880	0.0095700	-0.81(NT)	0.00 (NB)	905.01
RRF	3	477.59	0.939	0.0006559	0.0050000	-0.85 (NT)	1.00 (B)	835.11
PGF	3	319.39	0.987	0.0002273	0.0001719	-0.81 (NT)	1.00 (B)	834.81
TQW	3	433.50	0.520	0.0006623	0.004826	-0.80 (NT)	0.00 (NB)	834.81
PL	2	228.31	0.811	0.0002038	0.0000187	-0.80 (NT)	0.28 (B)	834.91
QSRKF	5	664.82	0.578	0.0006412	0.0104700	-0.59 (NT)	0.00 (NB)	850.71
SGF	3	309.35	0.947	0.0015770	0.0179400	-0.83 (NT)	0.03 (NB)	832.51
ADAF	4	422.47	0.708	0.0007727	0.1216000	-0.88 (NT)	0.97 (B)	834.81
GRGW	4	474.57	0.959	0.0014720	0.1128000	-0.74 (NT)	1.00 (B)	834.91
APHW	4	509.61	0.917	0.0000995	0.0018480	-0.84 (NT)	0.50 (NB)	834.71
AF	2	236.28	0.973	0.0003628	0.0041230	-0.80 (NT)	0.99 (B)	834.81
NPL	3	342.43	0.604	0.0004216	0.0002498	-0.82 (NT)	0.00 (NB)	834.81
GRL	3	344.45	0.775	0.0043090	0.0339300	-0.80 (NT)	0.99 (B)	830.31
SAF	3	323.37	0.820	0.0009077	0.0088550	-0.80 (NT)	0.00 (NB)	834.61
RSSF	4	495.57	0.578	0.000940	0.0165400	-0.78 (NT)	0.00 (NB)	834.91

* NT : Non-Toxic, NB : Non-Bitter, B : Bitter

The enzyme-substrate interaction of α-amylase is influenced by the three domains. The carboxyl terminus of domain B connects it to the majority of the domains in domain A, which contains the substrate-binding sites. While domain B is in charge of maintaining both substrate selectivity and enzyme stability, domain C is in charge of maintaining the stability of the enzyme's catalytic regions [44], [45]. Research on the α-amylase enzyme shows that its catalytic activity involves seven major amino acid residues consisting of TRP59, TRP58, TYR62, HIS299, ASP96, HIS305, ASP197, and ASP300, suggesting that these amino acids can inhibit the α-amylase enzyme and peptide binding to these residues may control the metabolism of carbohydrates [46]. According to Table 4, peptide candidates with *p*-values lower than 0.05 were predicted to interact with peptide-binding with α-amylase (PDB ID: 2QV4). Dipeptide and tripeptide PL, AF, GCL, PHY, PKF, PGF, and SAF maintained similar binding patterns, interacting with TRP58, TRP59, ASP300, and HIS305. According to MacGregor *et al.,* these residues play a role in proton transport and substrate stacking [47]. The most efficient inhibitors of α-amylase in dipeptide and tripeptide groups were RF and RRF, which attached to 6 and 7 crucial amino acid residues, respectively. In contrast, the tetrapeptide APHW revealed

interactions with 4 key amino acid residues and was demonstrated to be the best α-amylase inhibitor in its group. However, longer peptides, including QPKGL, QSRKF, GRPQPRL, and PGCAETF showed multi-point interactions with peripheral binding residues (GLN63, TYR52, and TRP357) as well as catalytic residues. These extended contacts may increase binding affinity and specificity, supporting their potential as highly potent inhibitors. Additionally, these four peptides blocked five key residues of α-amylase and demonstrated a strong binding ability to α-amylase.

Table 3: The binding potential results from PepSite2 for bioactive peptide candidates and α-glucosidase (2QMJ) as the receptor.

Peptide Length	Peptide No.	Peptide Sequence	Bound Residues of α-glucosidase (2QMJ)
2	1.	RF	ASP327*, ILE364, TRP406*, TRP441*, ASP443*, MET444, ARG526*, ASP542*, PHE575*, H IS600*
	2.	EF	ASP327*, ILE364, TRP406*, ASP443*, MET444, ARG526*, ASP542*, PHE575*
	3.	RL	TYR299*, TRP406*, ARG526*, ASP542*, PHE575*
	4.	PL	TYR299*, TRP406*, ARG526*, ASP542*
	5.	AF	ASP327*, ILE364, TRP406*, ASP443*, MET444, ASP542*, ARG526*
3	6.	GCL	TYR299*, ASP327*, ILE364*, TRP406*, ASP542*, PHE575*, HIS600*
	7.	PHY	TYR299*, ASP327*, ILE364, TRP406*, TRP441*, ASP443*, MET444, ASP542*, HIS600*
	8.	PKF	ASP327*, ILE364, TRP406*, TRP441*, ASP443*, MET444, ARG526*, ASP542*, PHE575*, HIS600*
	9.	RRF	ASP327*, ILE364, TRP406*, TRP441*, ASP443*, MET444, ARG526*, ASP542*, PHE575*, HIS600*
	10.	PGF	TYR299*, ASP327*, ILE364, TRP406*, ASP443*, MET444, PHE450*, ARG526*
	11.	TQW	TYR299*, ASP327*, ILE364, TRP406*, TRP441*, ASP443*, PHE575*, HIS600*
	12.	SGF	TYR299*, ASP327*, ILE328, ILE364, TRP406*, ASP443*, MET444, ARG526*
	13.	NPL	TYR299*, ASP327*, TRP406*, ASP542*, PHE575*
	14.	GRL	TYR299*, ASP327*, ILE364, TRP406*, ASP443*, ARG526*, ASP542*, PHE575*, HIS600*
	15.	SAF	TYR299*, ASP327*, ILE364, TRP406*, ASP443*, MET444, ARG526*, ASP542*, PHE575*, HIS600*
4	16.	ADAF	ASP327*, ILE364, TRP406*, ASP443*, MET444, PHE450*, ARG526*, ASP542*, PHE575*
	17.	GRGW	TYR299*, ASP327*, ILE328, ILE364, TRP406*, TRP441*, ASP443*, ASP542*, PHE575*, HIS600 *
	18.	APHW	TYR299*, ASP327*, ILE364, TRP406*, TRP441*, ASP443*, PHE450*, ARG526*, ASP542*, PHE575*
	19.	RSSF	TYR299*, ASP327*, ILE364, TRP406*, ASP443*, MET444, PHE450*, ARG526*, ASP542*, PHE575*
5	20.	QPKGL	TYR299*, ASP327*, ILE328, ILE364, TRP406*, ASP443*, PHE450*, ASP542*, PHE575*, HIS600*
	21.	QSRKF	TYR299*, ASP327*, ILE364, TRP406*, TRP441*, ASP443*, PHE450*, ARG526*, TRP539, ASP542*, PHE575*, HIS600*
7	22.	GRPQPRL	TYR299*, ASP327*, ILE328, ILE364, TRP406*, TRP441*, ASP443*, MET444, PHE450*, ARG526*, TRP539, ASP542*, PHE575*, HIS600*
	23.	DSNNPEF	TYR299*, ASP327*, ILE328, ILE364, TRP406*, TRP441*, ASP443*, MET444, PHE450*, ARG526*, TRP539, ASP542*
	24.	PGCAETF	ASP327*, ILE328, ILE364, TRP441*, ASP443*, MET444, PHE450*, ARG526*, TRP539, ASP542*, PHE575*, HIS600*

* Binding site of -glucosidase (2QMJ)

3.4 *Molecular docking of candidate peptides with α-glucosidase and α-amylase*

Molecular docking is a computer method that helps understand the interaction of biomolecules with small molecules, like drugs and peptides, which is important for potential therapeutic applications [48]. The main targets for type 2 diabetes treatment are α-glucosidase and α-amylase inhibition. Although the mechanisms of peptides inhibit these 2 enzymes are still unknown, it is accepted that inhibition happens when peptides attach to the active site of enzymes to interfere with the catalytic activity. We observed candidate peptides with the largest number of key amino acid residues from each group, which were chosen for further study using molecular docking

(Tables 3 and 4). Molecular docking was performed to evaluate enzyme binding energy and study the correlation between the interactions of the enzyme and peptide candidates. Acarbose, a well-known carbohydrate digestive enzyme inhibitor, was also utilized as a control to compare the interactions of the candidate peptide. We examined the docking parameters and redocked the cocrystallized ligand into its respective active site to find the optimal conditions. The overlay of the native ligand conformation before and after validation of the docking method revealed minimal structural deviation (Figure 2). Furthermore, the RMSD values for the native ligand were 1.89 Å for α-glucosidase and 1.30 Å for α-amylase, according to the docking method's validation analysis. All analyzed ligands exhibited RMSD values below 2 Å, validating docking accuracy and demonstrating strong alignment between computationally predicted and experimentally determined conformations [49]. The results indicate an 80% success rate in reproducing ligand binding conformations, accompanied by moderate correlations (r = 0.5–0.6) between computational predictions and experimental binding affinities [28]. These findings facilitate a comprehensive assessment of the docking protocol's reliability and its potential utility for lead compound optimization and subsequent investigations. To evaluate the binding ability of each ligand to the receptor, the analysis is based on binding affinity scores, hydrogen bonds, bond lengths, and residual interactions [50].

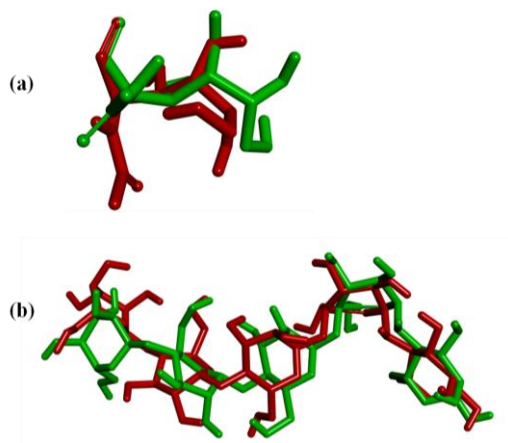

Figure 2: Overlay native ligand conformation PubChem CID 1738118 (a) and PubChem CID 24755467 (b) before validation (red) and after validation (green).

Table 4: The binding potential results from PepSite2 for bioactive peptide candidates and α-amylase (2QV4) as the receptor.

Peptide Length	Peptide No.	Peptide Sequence	Bound Residues of α-amylase (2QV4)
2	1.	RF	TRP58*, TRP59*, TYR62, ARG196, ASP197*, GLU233*, HIS299, ASP300*, HIS305*
	2.	RL	TRP58*, TRP59*, TYR62, ASP197*, GLU233*, HIS299, ASP300*
	3.	PL	TRP58*, TRP59*, ASP300*, HIS305*
	4.	AF	TRP58*, TRP59*, ASP300*, HIS305*
3	5.	GCL	TRP58*, TRP59*, ASP300*, HIS305*
	6.	PHY	TRP58*, TRP59*, ASP300*, HIS305*
	7.	PKF	TRP58*, TRP59*, ASP300*, HIS305*
	8.	RRF	PHE17, TRP58*, TRP59*, TYR62, ARG195*, ASP197*, GLU233*, HIS299, ASP300*, HIS305*
	9.	PGF	TRP58*, TRP59*, ASP300*, HIS305*
	10.	TQW	LYS261, GLU272, TYR276, TRP284, ASP300*, HIS305*
	11.	SGF	TRP58*, TRP59*, ASP300*, HIS305*
	12.	NPL	TRP58*, TRP59*, TYR62, GLU233*, HIS299, ASP300*, HIS305*
	13.	GRL	TRP59*, HIS305*, ASP356
	14.	SAF	TRP58*, TRP59*, ASP300*, HIS305*
4	15.	APHW	TRP58*, TRP59*, ASP300*, HIS305*, ASP356
	16.	RSSF	TRP58*, TRP59*, ASP300*, ASP356
5	17.	QPKGL	TYR52, TRP58*, TRP59*, ASP197*, HIS299, ASP300*, HIS305*
	18.	QSRKF	PHE17, TRP58*, TRP59*, TYR62, GLU233*, HIS299, ASP300*, HIS305*
6	19.	GRPQPRL	HIS15, PHE17, VAL42, SER43, PRO44, TRP58*, TRP59*, ASP96, ASP197*, HIS299, ASP300*, HIS305*
	20.	DSNNPEF	PHE17, TRP58*, TRP59*, TYR62, GLN63, HIS101, LEU165, HIS299, ASP300*, HIS305*
	21.	PGCAETF	PHE17, PRO54, TRP58*, TRP59*, TYR62, GLU233*, HIS299, ASP300*, HIS305*, ASP356, TRP357

* Binding site of α-amylase (2QV4)

Table 5: The results of docking scores, binding sites, interaction types and bond lengths of the different ligands with α-glucosidase (2QMJ) from AutoDock Vina.

Ligands	Binding Affinity (kcal/mol)	Hydrogen Bond	Electrostatic	Hydrophobic Interaction
RF	-8.0	ASP203* (2.62, 2.11 Å) ASP542* (2.49, 2.30 Å)	ASP443* (4.13 Å) ASP542* (3.42 Å)	TYP229* (2.58, 5.03 Å)
PKF	-7.0	THR205 (2.15 Å) ASP542* (2.22, 2.60, 2.25 Å) GLN603 (2.25, 2.59 Å)	ASP443* (4.08 Å) ASP542* (4.65 Å)	PHE575* (5.02 Å) ALA576 (4.46 Å)
RRF	-8.3	ASP327* (2.63, 2.74 Å) ASP443* (2.46, 2.66 Å) ASP542* (2.92, 3.00 Å) GLN603 (2.41, 2.80 Å) TYR605* (2.20 Å)	ASP327* (3.07 Å) ASP443* (4.17 Å)	TYP229* (4.04 Å) TRP406* (5.02 Å) PHE450* (3.70 Å) PHE575* (4.97 Å)
GRL	-8.1	ASP203* (2.32, 2.44, 2.60 Å) ASP542* (2.40, 3.04 Å)	ASP203* (3.37 Å) ARG526* (5.20 Å) ASP542* (5.47 Å)	TYP229* (4.78 Å)
SAF	-6.9	ASP203* (3.09, 1.98 Å)	ASP443* (4.10 Å)	TRP406* (5.14 Å) PHE450* (4.51 Å)
APHW	-7.5	ASP203* (2.80 Å) THR205 (2.25 Å) ARG526* (2.11 Å) ASP542* (2.99, 2.05 Å)	ARG526* (5.10 Å)	PHE450* (3.72, 3.99 Å) LEU473 (4.36 Å) ALA576 (4.79 Å)
QSRKF	-7.8	THR205 (2.72, 2.79 Å) TRP406* (2.58 Å) ASP542* (2.97, 2.11, 2.83 Å) ASP443* (2.45 Å)	-	PHE450* (3.68 Å) PHE575* (5.33 Å) ALA576 (4.11 Å)
GRPQPRL	-8.5	THR205 (2.57 Å) ASN207 (2.45 Å) ASN209 (2.14 Å) ASP443* (2.46 Å) ASP474 (3.74 Å) ARG526* (2.43 Å) THR544 (2.70 Å) ASP542* (2.56, 1.88 Å) HIS600* (2.10 Å) TYR605* (2.62, 2.19 Å)	ASP474 (3.07 Å) ARG526* (5.24 Å) HIS600* (4.85 Å)	TYP229* (4.59, 4.79 Å) ILE328 (4.29 Å) ILE364 (5.21 Å) TRP406* (4.47, 4.82 Å) LEU473 (4.83 Å) PHE575* (5.24 Å)
Acarbose	-6.6	ASP203* (2.23, 2.86, 2.51 Å) THR205 (2.54 Å) GLY208 (2.35 Å) ASP542* (2.80 Å) ASP549 (2.92, 2.70 Å)	-	-

*Amino acid residues that could play a role in inhibiting α-glucosidase upon interaction with the peptides.

Table 5 and Figure 3 exhibit the interaction between α-glucosidase and the candidate peptides. All candidate peptides showed strong binding affinities (-6.9 to -8.5 kcal/mol) and presented stronger binding than acarbose (-6.6 kcal/mol), indicating that the peptides are better inhibitors than acarbose. Peptides GRPQPRL, RRF, GRL, and RF displayed high binding energy at -8.5, -8.3, -8.1, and -8.0 kcal/mol, respectively (Table 5). All peptides formed multiple hydrogen bonds with essential residues in the active or nearby sites of the enzyme, making hydrogen bonding a crucial interaction mode. Common residues for hydrogen bonds included ASP203, ASP542, THR205, and ARG526, which are important for the catalytic activity and substrate binding of α-glucosidase [51], [52]. The peptide GRPQPRL generated 7 hydrogen bonds with several residues, such as ASP443, ARG526, ASP542, HIS600, and TYR605. In comparison, acarbose only formed 4 hydrogen bonds with ASP203 and ASP542, meaning the peptide had a broader interaction interface than acarbose (Table 5 and Figure 3). Furthermore, RRF, GRL, and RF were also connected to several key residues such as ASP327, ASP443, and GLN603, which are involved in substrate recognition and catalysis. The distance of hydrogen bonds plays a crucial role in the strength of peptide-enzyme specificity binding, suggesting

that bonds between 2.5 and 3.5 Å, as well as those shorter than 2.5 Å, are considered strong in binding affinity and specificity [53]. The results presented the excellent distance of hydrogen bonds that interacted between the enzyme and candidate peptides (Table 5). Additionally, electrostatic and hydrophobic interactions also support binding affinity. It plays a role in stabilizing peptide binding via charge complementarity, while the hydrophobic residues are preferentially near the catalytic cleft, helping to place the ligand correctly [43]. Table 5 and Figure 3 showed interactions of candidate peptides with negatively charged residues (ASP443, ASP542, and ASP203) and hydrophobic residues (TRP406, PHE450, and PHE575). These residues supported the peptides' stable binding. Thus, these factors all backed up the peptides' effectiveness as α-glucosidase inhibitors. Other α-glucosidase inhibitory peptides, like QSRKF and APHW, also showed a strong ability to inhibit α-glucosidase by forming four hydrogen interactions. However, PKF and SAF showed less potential because they only have one hydrogen bond. The docking results suggest that peptides such as GRPQPRL, RRF, GRL, and RF may serve as highly potent α-glucosidase inhibitors, offering stronger and more diverse interactions than the standard drug acarbose.

According to Siow *et al*., the mechanism of α-amylase showed that the inhibition process with peptides is based on their capacity to create a sliding barrier by hydrogen bonding with residues around the substrate-binding area [54]. Table 6 presents the results of docking scores, binding sites, interaction types and bond lengths of the different ligands with α-amylase. The candidate peptides RF, RRF, APHW, and acarbose showed strong binding energies at -8.0, -7.8, -8.7, and -8.2 kcal/mol, respectively. These results matched the number of important residues, showing that APHW and acarbose interacted with 9 residues each, while RF interacted with 8 and RRF with 6 residues (Table 6 and Figure 4). The findings demonstrated the strong inhibitory effect of candidate peptides with hydrophobic or aromatic amino acid residues at the C-terminal end. These findings are in accord with those of Fadimu *et al*., who found that SPRRF, AIPPGIPY, and MLLL had high affinity binding scores of -9.1, -8.4, and -8.2 kcal/mol, respectively [17]. Other peptides, QPKGL, GRPQPRL, PGCAETF, and QSRKF, showed lower affinity binding than acarbose, with scores of -6.7, -7.0, -7.2, and -7.5, respectively. Following Table 6 and Figure 4, effective candidate peptides formed multiple strong hydrogen bonds (less than 2.5 Å), especially with catalytically relevant residues such as ARG195, ASP197, GLU233, ASP300, and HIS305.

Besides, they also stabilized through interactions with residues such as ARG195, ASP197, HIS299, and GLU233. Moreover, the results presented hydrophobic interactions with residues TRP58, TRP59, TYR62, LEU162, ILE235, and HIS305 that contributed to pocket stabilization and ligand affinity [41]. According to the results, the APHW candidate peptide can be a promising α-amylase inhibitor because it showed the strongest binding and the highest binding affinities compared to the standard medication acarbose.

The peptides exhibited various binding interactions, as shown in Figures 3 and 4, which illustrate their docking with α-glucosidase (2QMJ) and α-amylase (2QV4), respectively. The peptide GRPQPRL comprises both polar (Q) and basic (R) residues. The presence of proline (P) might also influence peptide rigidity, as this amino acid forms multiple hydrogen bonds with key catalytic residues, such as ASP203 and ASP542. The length and flexibility of the peptide help it fit well in the binding pocket, leading to a strong connection with α-glucosidase. These binding mechanisms align with QSRKF, which includes charged residues (K and R) and polar residues (Q and S). The combination of polar and charged residues enhances specificity for the acidic environment of α-glucosidase. However, the peptides RF and RRF contain the aromatic residue (F) and the basic residue (R). Even though it is small, it forms hydrogen bonds and interacts electrically with some residues like ASP542 and GLN603, while also having non-polar interactions with residues like TYP229, TRP406, PHE450, and PHE575, which help to increase the blocking effect on α-glucosidase. Figure 3 explained that hydrogen bonding was the predominant interaction method for α-glucosidase. Peptides abundant in polar or charged residues establish persistent hydrogen bonds within the acidic catalytic region of α-glucosidase, therefore enhancing specificity. Electrostatic and hydrophobic interactions enhanced peptide stability and facilitated deeper binding. As a result, GRPQPRL and RRF mainly strongly connect with α-glucosidase by forming several hydrogen bonds with the important parts of the enzyme. However, Figure 4 revealed that peptide PGCAETF consisted of A mix of hydrophobic (A and

F) and polar (T and E) residues. The peptide likely binds in a peripheral or secondary pocket, reducing its overall inhibitory potential. It interacts with HIS305 and ASP300, and it has less extensive contact with the core catalytic residues compared to APHW. While APHW contained aromatic residue tryptophan (W) and basic histidine (H), it interacted and bonded effectively to ASP197, GLU233, ASP300, and HIS305, which are crucial catalytic residues of α-amylase. The aromatic side chains (W and H) support π–π stacking and hydrogen bonding, especially within the more hydrophobic and compact pocket of α-amylase. Therefore, APHW exhibited the strongest affinity for α-amylase. According to Figure 4, α-amylase demonstrated a significant binding of peptides at the active cleft, frequently establishing multiple hydrogen bonds. Additionally, hydrophobic interactions with residues such as TRP59, LEU162, and ALA198 contributed to enhanced stability. The differential interaction profiles show the relevance of peptide sequence, side chain chemistry, and spatial orientation in influencing enzyme specificity and inhibitory potency. Peptides GRPQPRL, RRF, and APHW exhibit significant potential as dual inhibitors and necessitate more *in vitro* and *in vivo* confirmation for advancement in antidiabetic therapy.

Numerous studies have demonstrated that laboratory tests using derived peptides from plant sources suppress the activity of α-glucosidase and α-amylase. The peptides EGEPKLP, KDDLRSP, TPELKL, and LDYGKL from shiitake mushrooms inhibited α-glucosidase with IC_{50} values of 499, 550, 452, and 696 μM, respectively [55]. Moreover, peptides YGF and GMCC, obtained from fermented spent coffee grounds, showed reductions in enzyme activity of 95.31% and 89.79%, respectively [25]. Identify the polar or charged residues of peptides that augmented α-glucosidase enzyme inhibition based on these data. The α-amylase inhibitory peptide MMFPH, which is abundant in hydrophobic residues and produced from quinoa, demonstrated 66% inhibition [56]. Additionally, the peptide LDQTPRVF, obtained from hydrolyzed soybean protein, had dual inhibitory effects, with IC_{50} values of 2.52 mM for α-glucosidase and 3.08 mM for α-amylase [57]. Therefore, the protein patterns from previous research revealed inhibition of the two enzymes that align with this study. Overall, peptides abundant in polar/charged residues, with adequate spacing, encourage α-glucosidase binding due to their acidic and open binding site. Nonetheless, the presence of aromatic residues, especially in short peptides (2-3 residues), enhances the support of enzyme inhibition. On the other hand, peptides with aromatic or hydrophobic residues that fit well into the tighter and more hydrophobic α-amylase site are more effective at blocking α-amylase. Moreover, the findings revealed several peptides with significant abilities to block two enzyme activities, underscoring SCG as a significant food waste resource with considerable potential for value-added applications. This work corresponds with sustainability objectives by supporting waste valorization. These outcomes collectively highlight the importance and influence of this study. Furthermore, a recent study by Ibrahim *et al*., demonstrated the effectiveness of *in silico* approaches for the design and screening of dual α-glucosidase and α-amylase inhibitors [58]. They identified the peptides SVPA and SEPA through computational prediction and evaluated them using *in vivo* assays in their work. The results showed that both peptides effectively inhibited the activity of the target enzymes, with IC_{50} values ranging from 0.79 to 5.92 mM, which demonstrated superior inhibitory activity compared to the standard drug, acarbose [58]. These findings underscore the reliability of *in silico* methodologies in identifying bioactive peptides with therapeutic potential. Therefore, this study applied computational tools to identify SCG-derived peptides with promising enzyme inhibitory profiles. Additionally, these results provide a solid basis for future research focused on scaling up peptide production and conducting *in vitro* and *in vivo* evaluations to confirm their therapeutic efficacy.

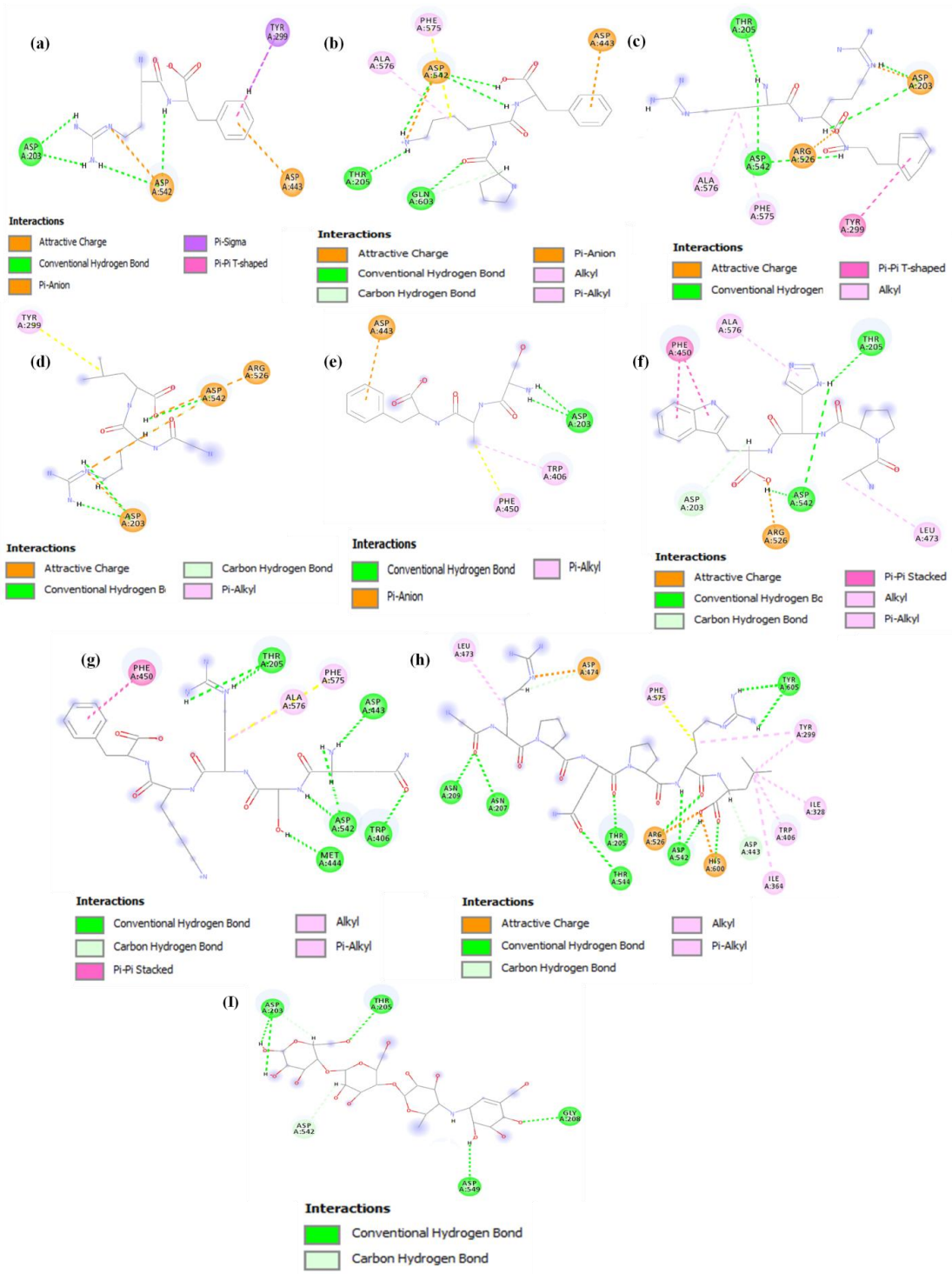

Figure 3: Two-dimensional representations of α-glucosidase (2QMJ) –ligand interactions at the relevant active sites of RF (a), PKF (b), RRF (c), GRL (d), SAF (e), APHW (f), QSRKF (g), GRPQPRL (h) and Acarbose (i).

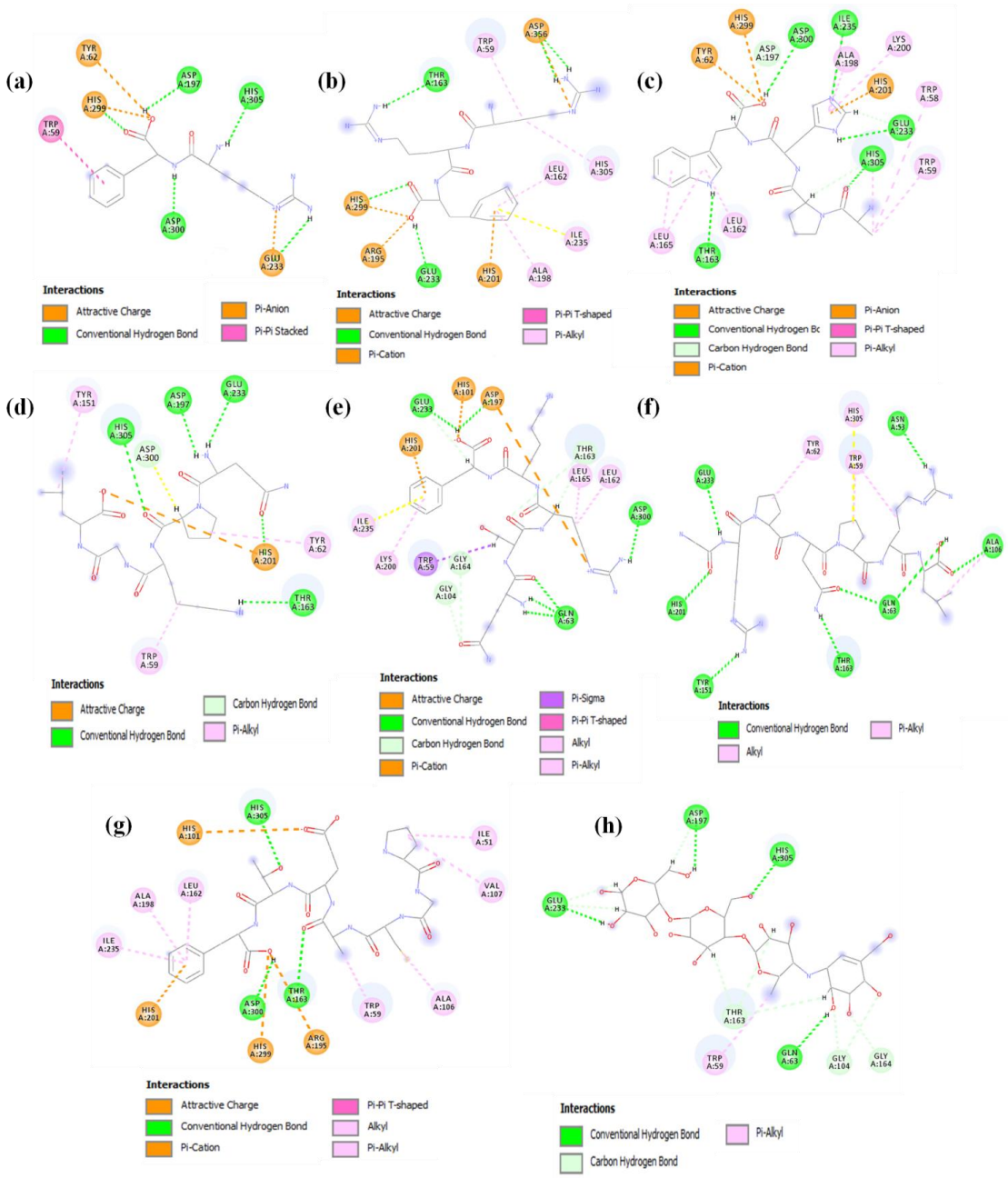

Figure 4: Two-dimensional representations of α-amylase (2QV4) –ligand interactions at the relevant active sites of RF (a), RRF (b), APHW (c), QPKGL (d), QSRKF (e), GRPQPRL (f), PGCAETF (g) and Acarbose (h).

Table 6: The results of docking scores, binding sites, interaction types and bond lengths of the different ligands with α-amylase (2QV4) from AutoDock Vina.

Ligands	Binding Affinity (kcal/mol)	Hydrogen Bond	Electrostatic	Hydrophobic Interaction
RF	-8.0	ASP197* (2.18 Å) GLU233* (2.29 Å) HIS299* (2.13 Å) ASP300* (2.42 Å) HIS305* (2.27 Å)	TYR62 (4.03 Å), GLU233* (4.73 Å) HIS299 (4.81 Å)	TRP59* (4.98, 5.47 Å)
RRF	-7.8	THR163 (2.38 Å) ARG195* (2.25 Å) GLU233* (2.09 Å) HIS299 (2.68 Å) ASP356 (2.35, 2.70 Å)	ARG195* (5.45 Å) HIS201 (3.94 Å) HIS299 (4.25 Å) ASP356 (5.58 Å)	TRP59* (3.68, 4.21 Å) LEU162 (4.84 Å) ALA198 (5.20 Å) HIS201 (4.94 Å) ILE235 (5.35 Å) HIS305* (5.08 Å)
APHW	-8.7	THR163 (2.30 Å) ASP197* (2.71 Å) GLU233* (2.43, 2.47 Å) ILE235 (2.61 Å) ASP300* (2.83 Å) HIS305* (2.10, 2.04 Å)	TYR62 (3.62 Å) HIS201 (3.70 Å) HIS299 (3.00 Å)	TRP58* (5.24 Å) TRP59* (4.54 Å) LEU162 (5.07 Å) LEU165 (4.88, 4.88 Å) ALA198 (5.08 Å) ALY200 (4.80 Å) HIS201 (4.58 Å) ILE235 (4.29 Å) HIS305* (4.34 Å)
QPKGL	-6.7	THR163 (2.30 Å) ASP197* (2.10 Å) HIS201 (2.11 Å) GLU233* (2.45 Å) ASP300* (2.12 Å) HIS305* (2.66 Å)	HIS201 (4.39 Å)	-
QSRKF	-7.5	GLN63 (2.14, 2.74, 2.39 Å), GLY104 (2.83 Å) THR163 (3.03, 2.63 Å) GLY164 (2.92 Å) ASP197* (2.17 Å) GLU233* (3.09, 2.66 Å) ASP300* (1.85 Å)	HIS101 (5.54 Å) ASP197* (4.96 Å)	TRP59* (2.58 Å) LEU162 (5.19 Å) LEU165 (4.54 Å) ALY200 (4.89 Å) HIS201 (2.51, 4.38 Å) ILE235 (4.24 Å)
GRPQPRL	-7.0	ASN53 (2.54 Å), GLN63 (2.19, 2.23 Å) ALA106 (2.95 Å) TYR151 (2.35 Å) THR163 (2.69 Å) HIS201 (2.30 Å) GLU233* (2.11 Å)	-	TRP59* (4.04, 4.68, 4.73 Å) TYR62 (5.49 Å) ALA106 (4.16 Å) HIS305* (5.03 Å)
PGCAETF	-7.2	THR163 (2.98 Å) HIS305* (2.23 Å) ASP300* (2.60 Å)	HIS101 (4.81 Å) ARG195 (5.45 Å) HIS299* (4.28 Å) HIS201 (2.84 Å)	ILE51 (4.67 Å) TRP59* (4.27, 3.69 Å) ALA106 (4.96 Å) VAL107 (4.31 Å) LEU162 (5.01 Å) ALA198 (5.39 Å) HIS201 (4.75 Å) ILE235 (4.98 Å)
Acarbose	-8.2	HIS305* (2.14 Å) GLN63 (2.50 Å) ASP197* (2.08, 3.06 Å) GLU233* (2.15 Å) GLY104 (2.38, 2.54 Å) GLY164 (2.36 Å) THR163 (2.72, 2.75, 2.76 Å) GLU233* (2.66, 3.00, 3.03 Å)	-	TRP59* (4.83, 4.25 Å)

*Amino acid residues that could play a role in inhibiting α-amylase upon interaction with the peptides.

4 Conclusions

This study uses computational algorithms to search for α-glucosidase and α-amylase inhibitors, two important enzymes involved in postprandial glucose management, to show the potential of bioactive peptides produced from SCG. Several peptides, including GRPQPRL and RRF, exhibited strong predicted α-glucosidase inhibitors with catalytic residues such as ASP203, ASP542, THR205, and ARG526, while peptide APHW showed excellent inhibition for α-amylase with ASP197, GLU233, ASP300, and HIS305 residues. The presence of specific amino acid residues, particularly aromatic and charged side chains, was associated with enhanced binding and inhibitory efficiency. Importantly, most peptides were predicted to be non-toxic and exhibited favorable blood stability, highlighting their suitability for therapeutic application. These results offer the basis for future *in vitro* and *in vivo* validation. Structural predictions in this study demonstrated approximately 80% accuracy, establishing a reliable basis for experimental scale-up. These findings validate both the computationally predicted bioactivity of SCG-derived peptides and their potential as effective antidiabetic agents. Collectively, the results indicate that SCG-derived peptides represent promising, cost-effective, and sustainable bioactive compounds with potential applications in antidiabetic drug development, nutraceuticals, and functional foods.

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Author Contributions

V.P.: investigation, methodology, research design, writing an original draft; M.S.: data curation, writing-reviewing and editing; V.P. and T.P. conceptualization, data analysis, writing-reviewing and editing, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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