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Article

ANTIHYPERTENSIVE AND ANTIDIABETIC PEPTIDES DERIVED FROM *IN SILICO* SIMULATED GASTROINTESTINAL DIGESTION OF QUINOA (*Chenopodium quinoa*) GLOBULINS AND MOLECULAR DOCKING STUDY

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This study evaluated the impact of *in silico* simulated gastrointestinal digestion (GID) of four quinoa globulins on the potential to release ACE and DPP-IV inhibitor peptides (antihypertensive and antidiabetic properties, respectively), as well as performed a molecular docking study to evaluate the interactions produced in the peptide-enzyme complexes. *In silico* GID performed on quinoa globulins resulted in the formation of amino acids as well as peptides with two to five residues. The peptides PSF, IPG, CSG, SPR, CSPG and PPN stood out for their high bioactivity scores (> 0.6), for not showing toxicity, as well as presenting potential inhibitory properties to both ACE and DPP-IV enzymes evaluated by ToxinPred, PeptideRanker and BioPep tools, respectively. The molecular docking analysis allowed highlighting that all peptides interacted with the enzymes, finding favorable binding energy values, different number and type of interactions, either at the level of the enzyme active sites or not, characteristics that together would define the potential of the established interaction of the complexes formed. The results, at the level of a first screening, support that GID of quinoa globulins can give rise to peptides with both antihypertensive and antidiabetic properties, requiring further *in vitro* and *in vivo* studies.

Keywords: quinoa globulins; simulated gastrointestinal digestion; *in silico*; molecular docking.

INTRODUCTION

Over the last two decades, food-derived bioactive peptides have attracted much attention for their potential to serve as natural alternatives or complements to synthetic drugs. Bioactive peptides (BP), embedded within the sequence of the precursor protein, can be released by gastrointestinal digestion and/or processing technologies. Once released, they have been demonstrated to exert a plethora of biological activities improving human health and reducing risk of chronic disorders.[1] Thus, BP have been evaluated for their antimicrobial, antihypertensive, antioxidant activities, blood-lipid-lowering effect, opioid role, anti-obesity, ability to bind minerals, antidiabetic, and antiaging effects/activities.[2] Evaluation of potential biological activities of food protein-derived BP involves different approaches including *in silico*, *in vitro* and *in vivo* studies. Due to the progress in the development of bioinformatics tools, the *in silico* approach is widely applied as a first step for pre-screening and it is later combined with the other two approaches.[3,4] *In silico* analysis has been greatly used to investigate the bioactive features of proteins and peptides, which is more economical and time-saving than the conventional method.[5] In the field of BP all the knowledge accumulated after two decades of identifying, isolating and testing peptides has been translated to mathematic algorithms for the development of *in silico* tools. Thus, PeptideRanker is a server that gives a peptide sequence a probability of being bioactive, based on a novel N-to-1 neural network algorithm. This server gives an overall bioactivity value, without considering specific bioactivities.[6] Meanwhile, ToxinPred was specifically developed to predict and design toxic/non-toxic peptides. Toxic peptides have been collected from various databases/studies and a model has been developed using the machine-learning technique support vector machine (SVM), for discriminating toxic peptides from non-toxic peptides.[7]

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In addition, quinoa, which is considered a pseudocereal, has been recognized as a complete food due to its protein quality. It has remarkable nutritional properties; not only from its protein content (15%) but also from its great amino acid balance.[8] Albumins and globulins represent the main storage proteins in quinoa. According to Dakhili *et al*.[9] the mature quinoa seed predominantly consists of 11S-type globulin called chenopodin, comprising about 37% of the total protein. In addition, Burrieza *et al*.[10] reported the presence of legumin-like proteins (both 11S and 13S globulins) generally much more abundant in the quinoa seeds of different genotypes evaluated than the vicilin-like proteins (7S globulins).

Several *in vitro* and *in vivo* studies have been carried out on BP obtained from quinoa proteins through the action of various proteases, where antioxidant, antihypertensive and antidiabetic properties have been especially explored,[1,5,11-18] including in the studies stages such as purification, identification, as well as their characterization at the bioactivity level. *In silico* studies have recently begun to be used as a strategy for the identification of quinoa BPs, such as the work of Guo *et al*.[5] who performed *in silico* proteolysis with papain, ficin and stem bromelain, evaluating the resulting BPs for their antihypertensive (ACE inhibition) and antidiabetic (DPP-IV inhibition) properties. Likewise, Valenzuela-Zamudio *et al*.[4] evaluated the action of the enzymes pepsin, trypsin and chymotrypsin on quinoa globulins, resulting in peptides with antidiabetic properties (inhibition of alpha-amylase, alpha-glucosidase and DPP-IV). To date, no investigations have been reported in an *in silico* setting where the combined antihypertensive and antidiabetic properties of peptides released from the gastrointestinal digestion (GID) of quinoa globulins have been evaluated. Thus, this study aimed to evaluate the antihypertensive properties referred to ACE inhibition and antidiabetic properties by inhibition of DPP-IV of the peptides found from gastrointestinal digestion of quinoa 11S and 13S globulin proteins simulated

in silico, as well as to evaluate by molecular docking the interactions established between the peptides and the key enzymes.

METHODOLOGY

Protein sequences

The quinoa globulin proteins used in the present study were: 11S seed storage globulin, 11S globulin seed storage protein 2-like, 13S globulin seed storage protein 1-like and the 13S globulin seed storage protein 2-like, taking into consideration a previous study by Guo *et al*.[5] The sequences in FASTA format of the four proteins were obtained from the NCBI database.[19]

Gastrointestinal digestion of quinoa globulin *in silico*

The gastrointestinal digestion of the four globulins was simulated simultaneously with the three main enzymes involved in this process, namely pepsin (pH > 2) (EC 3.4.23.1), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1), using the BIOPEP[20] platform according to Minkiewicz *et al*.[21] As a result of the simulated digestion, the fragments generated by each protein were obtained (Table 1). Subsequently, each peptide fragment consisting of 3 or more amino acids was evaluated for its bioactivity potential using the PeptideRanker[22] tool, obtaining values in the range from 0 to 1, with the most important results being those reaching values closer to 1. Fragments with values > 0.6 were considered as potentially bioactive as mentioned by Valenzuela-Zamudio *et al*.[4] The toxicity of the peptides was predicted using ToxinPred[23] according to Gupta *et al*.[7] Non-toxic peptides were evaluated for their possible antihypertensive (ACE inhibitor) and antidiabetic (DPP-IV inhibitor) properties using the BIOPEP[7] platform.

Molecular docking study

In silico molecular binding of peptides generated from quinoa globulin protein with the target enzymes ACE and DPP-IV were elucidated using a docking analysis. Briefly, first, the ligands (peptides) and target enzymes were prepared, the ligands were constructed manually with the Chimera[24] program and subsequently saved in PDB format. The crystal structures of human ACE in complex with lisinopril (PDB ID: 1O8A) and human DPP-IV in complex with a beta amino acid inhibitor (PDB ID: 1X70) were obtained from the Protein Data Bank.[25] Using AutoDockTools[26] all water molecules and other ligands were removed. Then polar hydrogens, Gasteiger charges and rotatable bonds were added to the prepared structures. The AutoDockTools[26] was used for docking assays of the peptides (ligands) within the catalytic cavity of ACE and DPP-IV enzymes. For both enzymes the docking grid was designed to encompass binding site residues. Then, the best interaction energies (lowest value) established between each ligand with each enzyme were obtained. PyMOL[27] was used to view the diagrams of each enzyme-peptide interaction, obtaining the residues (amino acids) of the enzymes with which each of the peptides interact at 5 Å supported from the Discovery Studio Visualizer[28] to identify potential ligand-enzyme interactions, such as hydrogen bonds, hydrophobic, electrostatic, and coordination interactions specifically located at the active sites. The molecular docking study was also performed for the drugs Lisinopril (antihypertensive drug related to ACE inhibition) and Sitagliptin (antidiabetic drug related to DPP-IV inhibition), both of which were used as positive controls.

RESULTS AND DISCUSSION

Evaluation of *in silico* gastrointestinal digestion of quinoa globulin

Quinoa globulins: 11S seed storage globulin, 11S globulin seed storage protein 2-like, 13S globulin seed storage protein 1-like and the 13S globulin seed storage protein 2-like, obtained under FASTA format from the NCBI database,[19] presented in their conformation a number of 479, 313, 463 and 542 amino acids, respectively, each of them with a particular conformation and amino acid sequence, the same that were employed in the simulation of gastrointestinal digestion (GID) analysis. Valenzuela-Zamudio *et al*.[4] indicate that this analysis is often used in bioactive studies, in which proteins are subjected to sequential hydrolysis; the resulting hydrolysate represents a pool of peptides resembling those generated during digestion of proteins in the human gastrointestinal tract. Also, Panjaitan *et al*.[29] indicate that BIOPEP is a tool to simulate enzymatic hydrolysis using certain proteases and to estimate the release of bioactive peptides; it also contains details of the structures of the bioactive peptides, as well as their probable bioactivity.[30] Simulated *in silico* GID of quinoa globulins using the BIOPEP[20] platform gave rise to diverse structures, corresponding to amino acids and peptides containing between 2 and 5 amino acids in number, the results are shown in Table 1.

The resulting peptides with a number of amino acids greater than or equal to 3 (a total of 57 peptides) were analyzed for their bioactivity potential using the PeptideRanker[22] tool, a database that provides certain classes of bioactive peptides with specific structural characteristics;[31] in addition to measuring the theoretical bioactivity of the peptides, presenting as score values of 0 (poorest bioactivity) and 1 (most likely to be bioactive).[5] From the PeptideRanker analysis, bioactivity values in the range between 0.032 and 0.960 (supplementary material, Table 1S) were found. Thus, the study was continued with all those peptides with a score > 0.6, being them in order of potential bioactivity SPF > PSF > CSPG > PPN > IPG > CSG > SPR and CSL (0.960-0.601), respectively. In addition, none of the eight peptides were considered toxic (Table 2), according to ToxinPred[23] tool. The potential ACE and DPP-IV inhibitory properties were explored in the eight selected peptides, resulting that the peptides PSF, IPG, SPR, CSPG and PPN presented both bioactive properties evaluated, while CSG only presented the property to inhibit ACE and SPF and CSL only to inhibit DPP-IV (Table 2).

The study by Valenzuela-Zamudio *et al*.[4] revealed the presence of DPP-IV inhibitory antidiabetic peptides from *in silico* hydrolysis (performed with pepsin, trypsin and chymotrypsin) in quinoa proteins, finding 23 fragments of high bioactivity potential, highlighting in this property: PF, PPG, PM, SW, IW, SF, PP, PPL, PG, PY, VW and PL, where several of the mentioned dipeptides have also been found in the present study (see Table 1). Guo *et al*.[15] in a study with a similar objective to the present study, except that the simulated GID was performed *in vitro* with a hydrolyzed quinoa protein concentrate using pepsin and pancreatin, were able to identify by ESI-Q-TOF-MS/MS, a total of 37 fragments, consisting of between 6 to 15 amino acids, where those with the highest score of potential bioactivity (> 0.8) and with ACE-inhibition properties were FHPFPR, NWFPLPR and NIFRPF. Similarly, Vilcacundo *et al*.,[1] after a hydrolysis similar to that performed by Guo *et al.,*[15] identified the IQAEGGLT peptide with DPP-IV inhibition properties. The different results found in the *in vitro* and *in silico* studies could be influenced by the type of enzymes selected and used in both hydrolysis.

From the study, the peptides with high bioactivity scores and with properties to inhibit both ACE and DPP-IV enzymes (PSF, IPG, SPR, CSPG and PPN), were subjected to molecular docking analysis

Table 1. Fragments of quinoa globulin proteins generated from simulated *in silico* gastrointestinal digestion

Protein	Fragments
11S seed storage globulin accession: AAS67037.1	M - A - K - ST - T - T - L - F - L - L - SCS - IA - L - VL - L - N - G - CM - G - Q - G - R - M - R - E - M - Q - G - N - E - CQ - ID - R - L - T - A - L - E - PT - Y - R - IQ - A - E - G - G - L - T - E - VW - D - T - Q - D - Q - Q - F - Q - CSG - VS - V - IR - R - T - IE - PN - G - L - L - L - PSF - T - SG - PE - L - IY - IE - Q - G - N - G - ISG - L - M - IPG - CPE - T - F - E - SM - SQ - E - SW - R - E - G - M - E - R - G - M - R - G - G - R - F - Q - D - Q - H - Q - K - IR - H - L - R - Q - G - H - IF - A - M - PA - G - VA - H - W - A - Y - N - SG - N - E - PL - VA - V - IL - ID - T - SN - H - A - N - Q - L - D - K - D - Y - PK - R - F - Y - L - A - G - K - PQ - Q - E - H - SR - H - H - H - R - G - G - E - SQ - R - G - E - H - G - SD - G - N - VF - SG - L - D - T - K - S - V - VQ - SF - G - VSE - D - IA - E - K - L - Q - A - K - Q - D - E - R - G - N - I - VL - VQ - E - G - L - H - V - IK - PPSSR - SY - D - D - E - R - E - Q - R - H - R - SPR - SN - G - L - E - E - T - ICSA - R - L - SE - N - ID - D - PSK - A - D - VY - SPE - A - G - R - L - T - T - L - N - SF - N - L - P - IL - SN - L - R - L - SA - E - K - G - VL - Y - R - N - A - IM - A - PH - Y - N - L - N - A - H - S - I - IY - G - VR - G - R - G - R - IQ - I - VN - A - Q - G - N - S - VF - D - D - E - L - R - Q - G - Q - L - V - V - VPQ - N - F - A - V - VK - Q - A - G - E - E - G - F - E - W - IA - F - K - T - CE - N - A - L - F - Q - T - L - A - G - R - T - SA - IR - A - M - P - VE - V - ISN - IY - Q - ISR - E - Q - A - Y - R - L - K - F - SR - SE - T - T - L - F - R - PE - N - Q - G - R - Q - R - R - E - M - A - A
11S globulin seed storage protein 2-like accession: XP_021733866.1	M - E - ID - L - SPK - Q - SQ - K - VY - G - G - D - G - G - SY - Y - T - W - SSSD - L - PM - L - A - E - A - K - VG - G - A - K - L - L - L - Q - PH - G - L - A - L - PSY - SD - SA - K - VA - Y - VL - H - G - K - G - R - A - G - I - VM - PE - A - T - K - E - K - V - VPL - R - K - G - D - A - L - A - L - PF - G - V - VT - W - W - F - N - D - G - D - G - E - L - V - VL - F - L - G - D - T - SK - A - H - R - SG - E - F - T - N - F - L - L - T - G - VG - SL - F - H - G - F - ST - E - F - VSR - A - W - D - L - K - E - E - E - VE - K - L - VK - A - Q - K - G - Q - G - I - VK - L - R - E - G - Q - SL - PA - A - SE - E - D - A - K - G - M - VF - N - CE - SA - PL - D - VD - VK - G - G - G - R - V - V - VL - T - D - K - N - L - PL - VG - E - VG - L - G - A - D - L - VR - ID - SA - SM - CSPG - F - SSD - SA - F - Q - VT - Y - V - IR - G - SG - R - VQ - V - VG - ID - G - R - R - VL - E - T - R - VK - A - G - CL - F - I - VPR - F - F - V - VSK - IA - D - G - D - G - M - E - W - F - S - I - IT - T - PQ - P - VF - SH - L - A - G - R - T - S - VW - K - A - L - SPQ - VL - E - A - SF - D - VSPE - M - E - K - L - F - R - SK - R - T - SD - A - IF - F - A - PPN
13S globulin seed storage protein 1-like accession: XP_021752233.1	M - A - F - T - T - T - N - N - N - A - L - L - F - W - VPL - CL - L - VF - L - ISPSL - A - Q - L - PL - L - Q - R - Q - PQ - Q - PR - G - Q - Q - W - Q - H - D - CD - IQ - Q - L - Q - A - A - E - PT - H - R - L - R - A - E - A - G - V - IE - VW - E - SN - SE - Q - F - R - CA - G - VA - A - VR - Y - V - IE - PK - G - L - L - L - PSY - T - N - A - PY - VT - Y - VT - Q - G - R - G - IQ - G - V - I - VPG - CPE - T - F - E - SPR - G - SG - SD - T - T - R - E - G - Q - R - D - Q - H - Q - K - VF - R - VQ - E - G - D - V - IG - SPA - G - V - VQ - W - T - Y - N - D - G - D - A - P - I - VS - VT - L - L - D - L - SN - PN - N - Q - L - D - L - N - F - R - SF - Y - L - A - G - D - PQ - G - G - Q - E - R - R - PK - E - VA - G - K - N - IF - N - G - F - D - D - E - M - L - A - D - A - F - N - VD - T - E - T - IR - SM - K - A - E - N - D - E - R - G - S - I - IR - VE - R - D - L - E - IL - SPE - W - D - D - T - E - E - E - R - T - R - R - L - N - G - L - E - Q - T - L - CSL - IF - K - Q - N - ID - R - PSL - A - D - VF - T - K - H - G - G - R - IN - T - L - N - G - H - K - L - PL - L - Q - Y - L - Q - L - S - VE - R - G - VL - Y - K - N - A - L - M - T - PH - W - N - IN - A - H - S - I - IY - IT - R - G - T - G - W - IQ - VA - R - E - N - G - R - L - VF - D - D - R - VQ - E - G - Q - L - L - V - VPQ - N - F - V - V - VK - K - A - E - Q - E - G - L - K - W - VSF - K - T - N - D - N - A - M - ISPL - A - G - K - L - SA - IR - G - M - PE - E - VL - M - N - SY - D - M - SR - D - E - VR - R - L - K - Y - G - R - E - E - L - SL - F - SPR - T - R - SF
13S globulin seed storage protein 2-like accession: XP_021752668.1	M - SR - VF - L - L - PL - A - L - T - L - L - IL - SPT - SL - A - Q - L - G - F - Q - L - G - Q - SPF - L - PSG - Q - SSPQ - H - SR - L - Q - R - G - Q - Q - A - L - N - D - CQ - IN - Q - L - SA - N - E - PS - IR - IQ - A - E - A - G - IT - E - VW - D - PK - E - Q - Q - E - F - Q - CA - G - VT - V - IR - R - E - IE - PK - G - L - L - L - PH - Y - N - N - A - PS - ISY - V - IR - G - R - G - L - L - G - L - SSL - G - CA - D - T - Y - E - SG - SPE - F - F - SE - E - SR - R - SE - R - F - E - E - SR - R - SE - R - G - SE - E - M - R - D - Q - H - Q - K - VR - R - F - H - K - G - H - V - IG - L - PA - G - VSK - W - VY - N - D - G - E - D - R - L - T - I - VT - L - Y - D - T - N - N - F - Q - N - Q - L - D - D - N - L - R - SF - F - L - A - G - N - PQ - G - R - G - G - D - Q - SG - R - Q - H - E - SSR - R - H - T - R - G - G - Q - E - E - M - G - Q - N - IL - SG - F - D - K - Q - L - L - A - D - A - F - E - VE - SD - T - ISK - IQ - G - E - N - D - D - R - G - A - I - IR - VE - SG - E - L - E - M - L - IPE - W - D - Q - E - E - Q - R - SE - R - H - H - R - G - G - G - SE - R - SE - E - E - E - R - SE - R - H - H - R - G - G - R - G - R - Q - SE - SSR - PH - N - G - IE - Q - T - L - CSA - R - L - S - VN - ID - N - PE - R - A - D - VF - N - PQ - G - G - R - L - T - N - IN - SN - K - L - P - IL - N - Y - L - R - L - SA - E - K - VN - L - Y - K - N - A - IM - T - PN - W - K - IN - A - H - S - I - VY - F - T - K - G - SG - R - VQ - IA - N - H - E - G - E - L - VF - D - D - M - VQ - E - G - Q - L - V - V - VPQ - N - F - V - VL - K - R - A - G - Q - D - G - L - E - W - VA - L - L - T - N - D - N - A - M - SSPL - A - G - R - ISA - IR - G - M - P - IE - V - VM - N - SY - K - L - SR - E - E - A - Q - R - L - K - Y - G - R - Q - E - L - S - VF - SPSK - R - SE - R - R - G - D - E - Y - A - I - V

to determine the interactions established between peptide-enzyme complexes. Additionally, the SPF sequence with a high bioactivity score and with the characteristic of only inhibiting DPP-IV was evaluated.

Molecular docking study

The results of the molecular docking performed on the peptides released from the GID of quinoa globulins simulated *in silico* with ACE and DPP-IV enzymes are presented in Table 3 and Figures 1 and 2. Firstly, it is observed that the best conformations established between the different peptides and the ACE and DPP-IV enzymes gave predicted interaction energy values (kcal mol^{-1}) ranging from −7.69 to −11.15 and from −6.43 to −8.99 kcal mol^{-1}, respectively. Low values of activation energies are desirable, since they would indicate a good interaction set up. The interaction energy values found for Lisinopril (reference antihypertensive) and Sitagliptin (reference antidiabetic) were −11.81 and −8.62 kcal mol^{-1}, respectively (Table 3), values that are very close to those determined for the peptides under study. Values ranged from −7.03 and −8.86 and, from −5.1 to −8.2 kcal mol^{-1}, have been reported for peptides obtained from quinoa when interacting with ACE and DPP-IV, respectively.[4,15]

Table 2. Bioactivity score of quinoa globulin peptides released from simulated *in silico* gastrointestinal digestion, their toxicity and potential antihypertensive and hypoglycemic properties

Protein	Peptides	Predicted bioactivity score[a]	Toxicity[b]	Possible activity[c]	ACE inhibitor sequences previously reported[c]	DPP-IV inhibitor sequences previously reported[c]
11S seed storage globulin	PSF	0.920	Non	ACE and DPP-IV inhibitor	SF	PS-SF
	IPG	0.696	Non	ACE and DPP-IV inhibitor	IP-PG	IP-PG
	CSG	0.642	Non	ACE inhibitor	SG	-
	SPR	0.617	Non	ACE and DPP-IV inhibitor	PR	SP-PR
11S globulin seed storage protein 2-like	CSPG	0.775	Non	ACE and DPP-IV inhibitor	PG	SP-PG
	PPN	0.728	Non	ACE and DPP-IV inhibitor	PP	PP-PN
13S globulin seed storage protein 1-like	SPR	0.617	Non	ACE and DPP-IV inhibitor	PR	SP-PR
	CSL	0.601	Non	DPP-IV inhibitor	-	SL
13S globulin seed storage protein 2-like	SPF	0.960	Non	DPP-IV inhibitor	-	SP-PF

[a]Data accessed from PeptideRanker.[22] [b]Possible toxicity obtained from ToxinPred.[23] [c]The possible bioactivities were obtained from BIOPEP[20] and attributed to the complete peptide or part of it.

Table 3 and Figures 1 and 2 show that the different peptides evaluated established interactions, to a greater or lesser extent, with the target enzymes. Tahir *et al*.[32] indicate that the residues that form part of the active site of ACE are Gln281, Glu411, His513, His383, Glu384, His387, Tyr523, His353, Glu162, Tyr520, Lys511, and Ala354, in addition Corradi *et al*.[33] point out that the Zn^{+2} ion is a cofactor of ACE that is partly responsible for the binding strength between this enzyme and its inhibitors, being important to establish interaction between the ligand (peptide) with this element. Also, with respect to the type of interaction of the peptide-enzyme

Table 3. Molecular docking interactions of peptides from quinoa globulins, Lisinopril and Stagliptin, and its interactions with residues of ACE and DPP-IV

Peptide	ACE		DPP-IV	
	Binding energy (kcal mol^{-1})	Site residues	Binding energy (kcal mol^{-1})	Site residues
Lisinopril	−11.81	His387*, His383*, Ala354*, His353*, His513*, Lys511*, Gln281*, Tyr523*, Glu411, Val380, Ala356, Zn^{+2}	-	-
Sitagliptin	-	-	−8.62	Tyr666&, His740&, Tyr662&, Asn710&, Glu205&, Glu206&, Arg358, Arg125, Phe208, Val207, Phe357
PSF	−11.15	Tyr523*, His387*, Ala354*, Glu384*, His383*, Arg522, His513, His353, Val380, Val518, Lys368, Asn70, Glu143, Val351, Ser516, Glu411, Phe512, Tyr523, Ser355, Zn^{+2}	−8.99	Trp563, Val575, Ala564, Gln527, Lys512, Val558, Phe559, Asn562, Ile529, Thr565, Arg560, Ser577, Lys554, Trp629, Trp627, Gly628, Gly632, Val546, Tyr547, Asp545, Tyr752
IPG	−7.69	His513*, Glu384*, His353*, Tyr520*, Ala354*, Phe527, Tyr523, Val380, Phe457, Zn^{+2}	−6.43	Glu205&, Glu206&, Tyr666&, Ser209, Arg358, Phe357
SPR	−7.81	Glu411*, Glu384*, His387*, Tyr523*, Ala356, His410, Asn70, Glu143, Trp357, Zn^{+2}	−6.54	Tyr662&, Tyr666&, Glu205&, Glu206&, Tyr547, Phe357, Ser552, Asn710, Arg669
CSPG	−10.47	Ala354*, His353*, His387*, Gln281*, Glu411*, Tyr523*, Tyr520*, Gln281*, Lys511*, His383*, Gln530, Asp45, Thr282, Val380, Lys454, Val379, Phe527, Ser355, Val518, Phe457, Ser526, Zn^{+2}	−7.14	Tyr752, Asp545, Trp627, Trp629, Gly628, Val546, Gly632, Tyr547, Gln527, Arg560, Val558, Lys512, Ile529, Ala564, Trp563, Val575
PPN	−11.03	Glu162*, Ala354*, Glu384*, His383*, His513*, Tyr520*, Lys511*, Gln281*, Cys370, Asp377, Val380, Gln369, Val380, His353, Tyr523, Glu411, His387, Val518,Phe512, Glu384, Ser355, Lys511, Zn^{+2}	−6.79	Tyr631&, Ser630&, Tyr662&, Glu206&, Arg125&, Glu205&, Tyr666&, Val656, Val711, Tyr547, Asn710, Phe357, Cys551, Gln553, Ser552, Pro550, Gly549, Trp659
SPF	NE	NE	−6.99	Tyr631&, Arg125&, Glu205&, Tyr662&, Ser630&, Tyr666&, Phe357, Ser209

*Residues of the enzyme active sites of ACE. &Residues of the enzyme active sites of DPP-IV. NE: Not evaluated.

complex it has been reported that the presence of hydrogen bonds between the peptides and ACE contributes greatly to the stability of enzyme-peptide complex, which is intimately linked to the inhibitory potency on ACE activity.[15] The results found indicate that all the ligands evaluated presented interactions with residues of the active site of ACE (highlighted with the symbol* in superscript) as well as with other residues (Table 3, Figure 1), highlighting in number of interactions in descending order among all the ligands evaluated GSPG > Lisinopril > PPN > PSF > IPG = SPR. Only the peptides IPG and SPR showed favorable interactions with Zn^{+2} in the same way as with Lisinopril. It is also observed that among the peptides, IPG established the highest number of hydrogen bridge interaction (4) with the active site of ACE followed by the ligands SPR, CSPG, PPN and Lisinopril (with 2 interactions all of them). Regarding DPP-IV it has been reported that residues Ser630, Asp708, Asn710, His740, Tyr631, Tyr662, Tyr666, Glu205, Glu206 and Arg125, are part of the active site of DPP-IV.[34] At this point, only peptides IPG, SPR, PPN and SPF interacted with the residues of the active site of DPP-IV

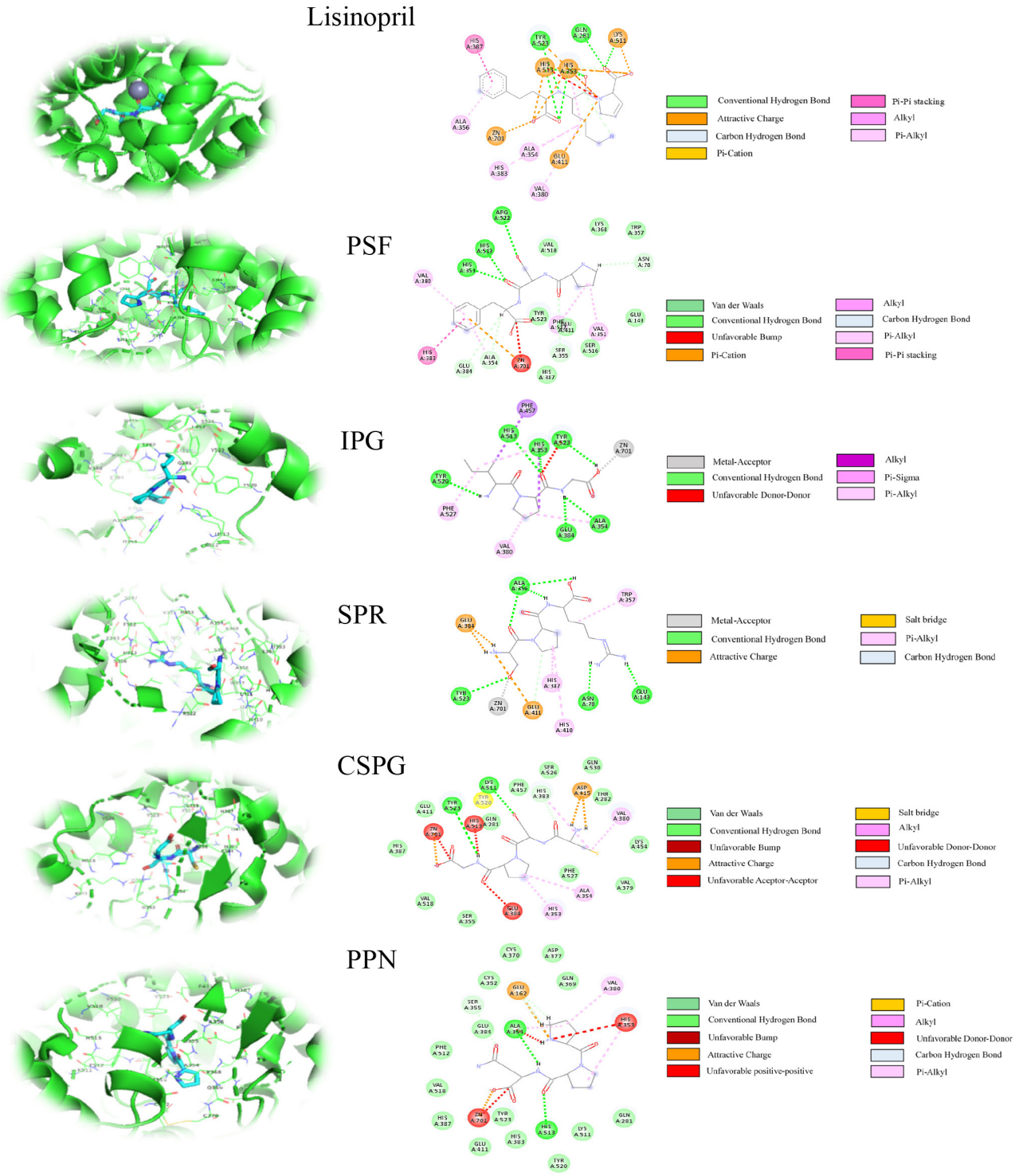

Figure 1. *Interaction between PSF, IPG, SPR, CSPG and PPN peptides with ACE enzyme (PDB 1O8A). On the right side is shown the 3D diagram of the ACE-peptides molecular interactions. On the left side is shown the 2D diagram of interactions obtained between peptides with ACE*

(Table 3 highlighted with the symbol & in superscript and Figure 2), as well as Sitagliptin; the rest bound to the enzyme at other sites. The ligands with the strongest interaction at the DPP-IV active site level were PPN > SPF > Sitagliptin = SPR > IPG.

The peptides interacting with the key binding pockets enzymes prevent their binding to the substrate, establishing what is known as a competitive inhibition pattern; but peptides can also be found that bind to different sites corresponding in this case to a non-competitive

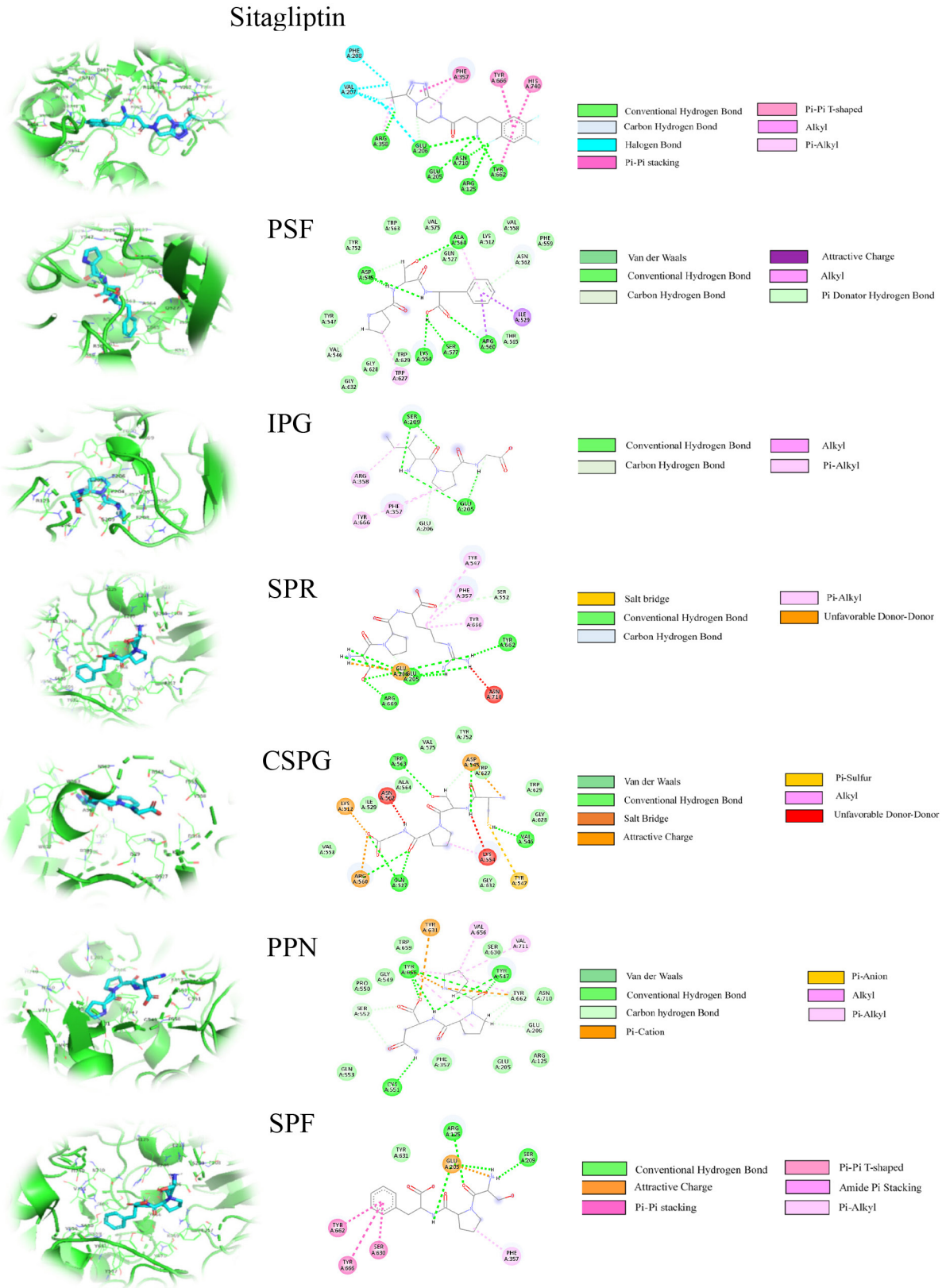

Figure 2. *Interactions between PSF, IPG, SPR, CSPG, PPN and SPF peptides with the enzyme DPP-IV (PDB 1X70). On the right side is shown the 3D diagram of the DPP-IV-peptides molecular interactions. On the left side is shown the 2D diagram of interactions obtained between peptides with DPP-IV*

inhibition[35] producing other changes in the enzyme so that it can no longer catalyze the reaction efficiently, therefore both types of interaction are important, affecting the catalytic reaction rate of the enzymes in different ways. Among the interactions identified between peptides-enzymes, hydrogen bond, attractive charge, hydrophobic, van der Waals interactions, among others, were found (Figures 1 and 2), as well as some unfavorable interactions were also evidenced, the latter does not necessarily mean that the peptide-enzyme complex is not stable, being necessary to verify this, to conduct molecular dynamics studies. Generally, different binding modes determine the inhibitory strength of enzyme activity.[36]

In addition to the results found and establishing a peptide structure-activity analysis, it has been stated that ACE prefers substrates or inhibitors (peptides) containing hydrophobic amino acid residues: Tyr (Y), Phe (F), Trp (W), Pro (P), or Lys (K) at the C-terminals, as well as Arg (R) residue.[37] When searching for these characteristics in the peptides found from the quinoa protein GID, it is observed that Pro is present in all peptides, in addition to Phe or Arg, in some of them (PSF and PSR). With respect to the effects of DPP-IV inhibition by peptides, the presence of Ala (A), Gly (G), Ile (I), Leu (L), Phe, Pro, Met (M), Trp, and Val (V) play an important role in determining the potency of DPP-IV inhibitory peptides,[38] a characteristic also evidenced in the peptides under study.

Based on the findings found and declared, the GID of quinoa globulin proteins evaluated under an *in silico* environment, generates peptides composed of three or four residues with antihypertensive and antidiabetic properties, peptides that could be present after the consumption of quinoa protein. The synthesis of these peptides and the evaluation of their effects in *in vitro* and *in vivo* models are in progress. On the other hand, the inhibitory potential of both ACE and DPP-IV enzymes by peptides will depend on a set of properties established between the complex formed, where the type of inhibition present (competitive or non-competitive), type and number of interactions established, as well as the participation of certain residues in the sequence of the peptide, among others, which evaluated as a whole, would play an important role in defining the efficacy of the biological activities.

CONCLUSIONS

The results of the present investigation showed that the product of gastrointestinal digestion to which fractions of quinoa globulin protein were subjected, simulated *in silico*, released peptides with a high potential of preceding bioactivity, among them peptides PSF, IPG, SPR, CSPG and PPN as ACE and DPP-IV inbititors, peptide CSG only as ACE-inhibitor and peptide SPF and CSL only as DPP-IV inhibitors. The molecular docking study allowed us to elucidate the different interactions established in type and number with the enzymes under study, supporting the bioactive potential of the peptides found. Further and deeper studies under *in vitro* and *in vivo* environments are needed to ensure the antihypertensive and antidiabetic potential of elucidated peptides.

SUPPLEMENTARY MATERIAL

The supplementary material (Table 1S) is available at http://quimicanova.sbq.org.br, as a PDF file, with free access.

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The authors declare no conflicts of interest.

REFERENCES

1. Vilcacundo, R.; Martínez-Villaluenga, C.; Hernández-Ledesma, B.; *J. Funct. Foods* **2017**, *35*, 531. [Crossref]
2. Akbarian, M.; Khani, A.; Eghbalpour, S.; Uversky, V. N.; *Int. J. Mol. Sci.* **2022**, *23*, 1445. [Crossref]
3. Imai, K.; Ji, D.; Nwachukwu, I.; Agyei, D.; Udenigwe, C. C. In *Comprehensive Foodomics*; Cifuentes, A., ed.; Elsevier: Amsterdam, 2021, p. 482. [Crossref]
4. Valenzuela-Zamudio, F.; Hidalgo-Figueroa, S. N.; Ortíz-Andrade, R. R.; Hernández Álvarez, A. J.; Campos, M. R. S.; *Food Chem.* **2022**, *394*, 133479. [Crossref]
5. Guo, H.; Richel, A.; Hao, Y.; Fan, X.; Everaert, N.; Yang, X.; Ren, G.; *Food Sci. Nutr.* **2020**, *8*, 1415. [Crossref]
6. Arrutia, F.; Fernández, R.; Menéndez, C.; González, U. A.; Riera, F. A.; *Sci. Rep.* **2017**, *7*, 17250. [Crossref]
7. Gupta, S.; Kapoor, P.; Chaudhary, K.; Gautam, A.; Kumar, R.; *PloS One* **2013**, *8,* e73957. [Crossref]
8. James, L. E. A.; *Adv. Food Nutr. Res.* **2009**, *58*, 1. [Crossref]
9. Dakhili, S.; Abdolalizadeh, L.; Marzieh Hosseini, S.; Shojaee-Aliabadi, S.; Mirmoghtadaie, L.; *Food Chem.* **2019**, *299*, 125161. [Crossref]
10. Burrieza, H. P.; Rizzo, A. J.; Vale, E. M.; Silveira, V.; Maldonado, S.; *Food Chem.* **2019**, *293*, 299. [Crossref]
11. Aluko, R. E.; Monu, E.; *J. Food Sci.* **2003**, *68*, 1264. [Crossref]
12. Nongonierma, A.; Le, M.; Dubrulle, C.; Barre, C.; Fitzgerald, R.; *J. Cereal Sci.* **2015**, *65*, 112. [Crossref]
13. Chirinos, R.; Pedreschi, R.; Velásquez-Sánchez, M.; Aguilar-Galvez, A.; Campos, D.; *Cereal Chem.* **2020**, *97*, 949. [Crossref]
14. Mudgil, P.; Kilari, B. P.; Kamal, H.; Olalere, O. A.; FitzGerald, R. J.; Gan, C. Y.; Maqsood, S.; *J. Cereal Sci.* **2020**, *96*, 103130. [Crossref]
15. Guo, H.; Hao, Y.; Richel, A.; Everaert, N.; Chen, Y.; Liu, M.; Yang, X.; Ren, G.; *J. Sci. Food Agric.* **2020**, *100*, 5569. [Crossref]
16. You, H.; Wu, T.; Wang, W.; Li, Y.; Liu, X.; Ding, L.; *Food Res. Int.* **2022**, *156*, 111176. [Crossref]
17. González-Muñoz, A.; Valle, M.; Aluko, R. E.; Bazinet, L.; Enrione, J.; *Food Sci. Hum. Wellness* **2022**, *11*, 1650. [Crossref]
18. Abbasi, S.; Moslehishad, M.; Salami, M.; *Int. J. Biol. Macromol.* **2022**, *213*, 602. [Crossref]
19. National Library of Medicine, https://www.ncbi.nlm.nih.gov/, accessed in October 2023.
20. Biochemia, https://biochemia.uwm.edu.pl/biopep/start_biopep.php, accessed in October 2023.
21. Minkiewicz, P.; Iwaniak, A.; Darewicz, M.; *Int. J. Mol. Sci.* **2019**, *20*, 5978. [Crossref]
22. Distill Deep, http://distilldeep.ucd.ie/PeptideRanker/, accessed in October 2023.
23. Toxin Pred, http://crdd.osdd.net/raghava/toxinpred/, accessed in October 2023.
24. *Chimera*, version 1.15; UCSF Resource for Biocomputing, Visualization, Informatics (RBVI) and NIH, San Francisco, CA, USA, 2020.
25. Protein Data Bank, https://www.rcsb.org/, accessed in October 2023.
26. *AutoDockTools 1.5.6*; The Scripps Research Institute, La Jolla, CA, USA, 2014.
27. *PyMOL*, version 2.4; Schrödinger Inc., LLC, New York, NY, USA, 2020.
28. *Discovery Studio Visualizer*, version17.2.0.16349; D. S. Biovia, San Diego, CA, USA, 2017.

29. Panjaitan, F. C. A.; Gomez, H. L. R.; Chang, Y. W.; *Molecules* **2018**, *23*, 2910. [Crossref]
30. Nongonierma, A. B.; FitzGerald, R. J.; *Food Chem.* **2014**, *165*, 489. [Crossref]
31. Qiao, M.; Tu, M.; Chen, H.; Mao, F.; Yu, C.; Du, M.; *Int. J. Mol. Sci.* **2018**, *19*, 2100. [Crossref]
32. Tahir, R.; Bashir, A.; Yousaf, M. N.; Ahmed, A.; Dali, Y.; Khan, S.; Sehgal, S.; *PloS One* **2020**, *15*, e0228265. [Crossref]
33. Corradi, H.; Chitapi, I.; Sewell, B.; Georgiadis, D.; Dive, V.; Sturrock, E.; Acharya, K.; *Biochemistry* **2007**, *46*, 5473. [Crossref]
34. Aertgeerts, K.; Ye, S.; Tennant, M. G.; Kraus, M. L.; Rogers, J. O. E.; Sang, B. C.; Skene, R. I.; Webb, D. R.; Prasad, G. S.; *Protein Sci.* **2004**, *13*, 412. [Crossref]
35. Wei, Y.; Liu, Y.; Li, Y.; Wang, X.; Zheng, Y.; Xu, J.; Sang, S.; Liu, Y.; *Nutrients* **2022**, *14*, 2420. [Crossref]
36. Huang, P. K.; Lin, S. R.; Chang, C. H.; Tsai, M. J.; Lee, D. N.; Weng, C. F.; *Sci. Rep.* **2019**, *9*, 15585. [Crossref]
37. Murray, B. A.; Fitzgerald, R. J.; *Curr. Pharm. Des.* **2007**, *13*, 773. [Crossref]
38. Nongonierma, A. B.; FitzGerald, R. J.; *J. Food Biochem.* **2019**, *43*, e12451. [Crossref]