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Original Article

In Silico Evaluation of Antimicrobial Potential of Amaranth, Chia and Quinoa Peptides Released During the Simulated Gastric Digestion and Their Effects on *Helicobacter pylori*

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HIGHLIGHTS

- *H. pylori* is a common infection and the leading cause of gastroduodenal pathologies.
- Gastric digestion of amaranth, chia, and quinoa proteins with pepsin revealed antimicrobial and antibiofilm peptides that may be effective against *H. pylori* growth.
- One particular peptide SWKYSHRRHHSNTGSL had high docking energy scores when docked to the three enzymes of *H. pylori.*

ABSTRACT

Helicobacter pylori is the most common cause of peptic ulcers and gastroduodenal pathologies and has been identified by the World Health Organization as a serious threat to human health. The increasing antibiotic resistance of *H. pylori* necessitates prevention and early intervention, as well as the discovery of novel drugs. Amaranth, chia, and quinoa are classified as pseudocereals and are known as superfoods because of their nutritional density. The effect of consuming these pseudocereals at the onset of *H. pylori* infection was investigated using *in silico* methods. 34 proteins from amaranth, chia, and quinoa were subjected to *in silico* pepsin digestion, and antimicrobial, antibiofilm, and cell-penetrating activities of the released peptides were analyzed. Peptides predicted to be cell-penetrating were further used for peptide-protein docking. 58 peptides were predicted to have antimicrobial activity whereas 76 were predicted to have antibiofilm activity. A total of 116 peptides were classified as cell-penetrating peptides, and those with the highest scores were used for peptide-protein docking with shikimate dehydrogenase, type II dehydroquinase, and D-alanine-D-alanine ligase of *H. pylori* to evaluate their enzyme inhibition potential. A peptide released from the chia seed proteins A0A1Z1EC55 and A0A1Z1EC46 with the sequence SWKYSHRRHHSNTGSL gave the highest docking energy scores for all three enzymes. To the best of our knowledge, this is the first work concerning the effect of ingested food on H. pylori infection and we believe that our results will provide valuable data and a new point of view for the scientists interested in this topic.

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Introduction

Pseudocereals are non-grass species that can be consumed in the same manner as cereals. In addition to being gluten-free, pseudocereals are rich in protein, fiber, and micronutrients, which are essential for maintaining a healthy lifestyle. Amaranth, quinoa, chia, buckwheat, album, and wattle seeds are the most common pseudocereals (Rao and Poonia, 2023). A comparison of the protein content of pseudocereals and cereals shows that pseudocereals have a higher protein content and are rich in essential amino acids. The storage proteins of pseudocereals are globulins, albumins, glutellins, and prolamins, and their quantities vary by species. (Kaur et al., 2023). Amaranth *(Amaranthus hypochondriacus* L.), the sacred plant of Aztecs, Mayans, and Incas, is a member of the Amaranthaceae family of approximately 70 species. It appears in various local cuisines with seeds as a grain or its leaves as a vegetable. Indeed, the protein ratio of amaranth (%18) not only surpasses that of conventional cereals but also its major protein albumin provides all the essential amino acids crucial for healthy metabolism. Globulin 11S, amarantin, is the principal protein in amaranth isolates consisting of 501 amino acids. Globulin 7S on the other hand, is found in lower quantities and has not been sufficiently studied compared to 11S (Montoya-Rodríguez et al., 2015) Besides being a protein-rich plant, amaranth is a potential source of bioactive peptides. Peptides with antihypertensive, antithrombotic, antioxidant, angiotensin-converting enzyme (ACE) inhibition, immunomodulatory, and protease inhibition activities have been reported (Tovar-Pérez et al., 2019; Ayala-Niño et al., 2022; Zhu, 2023) Chia (*Salvia hispanica* L.), which is native to northern Guatemala and southern Mexico, belongs to the family Lamiaceae. Chia seeds are high in protein (18.9%), which reveals bioactive peptides, most of which possess dipeptidyl peptidase IV and ACE inhibition activities when digested with proteases (Grancieri et al., 2019). Another pseudocereal, quinoa (*Chenopodium quinoa Willd*.) is a member of the Chenopodiaceae family and is endemic to South America. The average protein content of quinoa is %15 and it contains all the essential amino acids and is rich in thionic amino acids and lysines (Navruz-Varli and Sanlier, 2016) Furthermore, quinoa proteins are precursors of bioactive peptides with various activities such as antidiabetic, antihypertensive, antioxidant and antiinflammatory (Guo et al., 2023).

Helicobacter pylori is a Gram-negative S-shaped bacterium that is estimated to infect nearly half of the population. It frequently causes chronic gastritis, and some individuals may develop gastroduodenal pathologies. Approximately 90% of gastric cancer cases are caused by *H. pylori* infection. It penetrates the gastric mucus layer with its flagella, produces urea for colonization, and adheres to gastric epithelial cells with adhesins. (Malfertheiner et al., 2023). *H. pylori* is most commonly transmitted via fecal-oral, oral-oral, or gastro-oral routes, in addition to routes other than person-to-person contact, such as wastewater, zoonotic, or ingestion (Mezmale et al., 2020). Conventional therapy for *H. pylori* infection includes proton pump inhibitors, amoxicillin, and clarithromycin. Due to antibiotic resistance, the World Health Organization (WHO) identified *H. pylori* as one of the twenty pathogenic bacteria that pose a serious threat to human health in 2017 (Al-Fakhrany and Elekhnawy, 2023). Common antibiotics used in treatment target cell wall, protein, or DNA biosynthesis; however, novel narrow-spectrum drugs have proven successful while sparing the gut microbiome. (Vita et al., 2022). In this study, we investigated the effect of bioactive peptides released during the gastric digestion of amaranth, quinoa, and chia seed proteins on the growth of *H. pylori* using *in silico* methods. Using in silico subtractive proteomics, Ibrahim et al. identified potential druggable targets that are not homologous to the human proteome or similar to the gut microbiome (Ibrahim et al., 2020). Among these 17 enzymes, we selected shikimate dehydrogenase, type II dehydroquinase, and D-alanine-D-alanine ligase as targets for the peptides released during the gastric digestion of pseudocereals. In addition to the inhibitory effect of the released peptides on the target enzymes of *H. pylori*, peptides with antimicrobial and antibiofilm activities were also investigated using *in silico* methods.

Materials and Methods

Protein sequences and simulated gastric digestion

Amaranth (*Amaranthus hypochondriacus*), quinoa (*Chenopodium quinoa*), and chia (*Salvia hispanica*) seed proteins (Table 1) were retrieved from the Uniprot database (The UniProt Consortium, 2023) and The National Center for Biotechnology Information (NCBI) (Sayers et al., 2022) according to (Montoya-Rodríguez et al., 2015; Burrieza et al., 2019; Grancieri et al., 2019; Valenzuela Zamudio et al., 2022). Gastric digestion of proteins with pepsin (EC 3.4.23.1) was simulated using the enzyme action tool of BIOPEP-UWM (Minkiewicz et al., 2019). Peptides with 5 or more amino acids were selected for further analyses.

Table 1. Proteins of amaranth, chia and quinoa used in simulated gastric digestion

Organism	ID	Protein name	AA
Amaranth	Q38712	11S globulin seed storage protein	501
	Q0GPA4	Cysteine proteinase inhibitor	247
	Q8S390	Seed protein AmA1	304
	J9PE35	ADP-glucose pyrophosphorylase	390
	X4Y205	Glycosyltransferase	487
	D6RSA4	Starch synthase	606
	A7LIU5	Acetolactate synthase	669
	Q8LL67	Polyamine oxidase	496
	F8RNK2	RING-type E3 ubiquitin transferase	173
	Q38719	Agglutinin	304
	A2I9A6	11S globulin	487
Chia	XP_047958388.1	11S globulin-like	453
	XP_047974399.1	11S globulin seed storage protein 2-like	431
	A0A1Z1EC46	Fatty acid desaturase 2 isoform 1	383
	A0A1Z1EC55	Fatty acid desaturase 2 isoform 2	383
	A0A1Z1EC53	Fatty acid desaturase 3 isoform 1	393
	A0A1Z1EC60	Fatty acid desaturase 3 isoform 2	383
	A0A1Z1EC64	Fatty acid desaturase 7 isoform 1	440
	A0A1Z1EC52	Fatty acid desaturase 8	429
	A0A2R4LNR5	Tubulin beta chain	450
	A0A2R4LNR9	Glyceraldehyde-3-phosphate dehydrogenase	337
	A0A2R4LNR7	Clathrin adaptor complex	438
	Q36769	Ribulose bisphosphate carboxylase large chain	473
	A0A0F6PN28	Oleosin	142
Quinoa	XP_021755284.1	11S globulin seed storage protein 2-like	355
	Q06AW2	11S seed storage globulin A	480
	Q06AW1	11S seed storage globulin B	479
	XP_021768828.1	Legumin A-like	480
	XP_021752668.1	13S globulin seed storage protein 2-like	542
	XP_021752233.1	13S globulin seed storage protein 1-like	463
	XP_021746146.1	Vicilin-like seed storage protein At2g28490	559
	XP_021758596.1	2S albumin-like	142
	XP_021764389.1	Vicilin-like antimicrobial peptides 2-2	560
	XP_021717850.1	Vicilin-like seed storage protein At2g18540	804

Antimicrobial and biofilm inhibition potentials of the peptides

Antimicrobial peptides (AMPs) are short sequences of amino acids (10-55 aa), that can be produced either by the innate immune system or released from food proteins during their gastrointestinal digestion. They mostly possess antimicrobial activity by disrupting the cell membranes of microorganisms through electrostatic interactions, leading to permeabilization of the cell membranes (López-garcía et al., 2022; Nayab et al., 2022). Peptides with a length of 10 or more amino acids were selected, and their antimicrobial activities were predicted using AMP Scanner which is a deep neural network (DNN)-based classifier (Veltri et al., 2018). One of the underlying reasons for *antibiotic resistance in H. pylori* is its ability to form a biofilm structure consisting of extracellular polymeric substances secreted by the organism that adhere to each other after colonizing the gastric mucosa (Hou et al., 2022). The antibiofilm activity potential of the peptides was analyzed using the free online server dPABBs choosing the Support Vector Machine (SVM) model (Sharma et al., 2016). Peptides with a threshold score of 0.5 and above were accepted as antibiofilm active. dPABBs server was also used to show the physicochemical properties of the released peptides.

Prediction of the cell-penetrating peptides and peptide-protein docking studies

MLCPP 2.0, an online web server that provides ML-based prediction of cell-penetrating peptides (CPP) with their uptake efficiency according to peptide sequence and physicochemical parameters was used to predict cell penetration probability of the peptides (Manavalan and Patra, 2022). Peptides with the highest CPP and uptake activity were selected for peptide-protein docking with shikimate dehydrogenase (PDB ID: 3MUF), type II dehydroquinase (PDB ID: 2XD9), and D-alanine-D-alanine ligase (PDB ID: 2PVP) of *H. pylori*. The protein structures of the enzymes were downloaded from the RCSB Protein Data Bank (PDB) (Berman et al., 2000) and the structures of the selected peptides were predicted using ColabFold (Mirdita et al., 2022). The target proteins were prepared for docking using UCSF ChimeraX (Pettersen et al., 2021; Meng et al., 2023). HPEPDOCK

web server was used for flexible peptide-protein docking (Zhou et al., 2018). The interactions between the peptides and targets were visualized using BIOVIA, Dassault Systèmes (BIOVIA Discovery Studio, 2024 Client, San Diego, CA), and UCSF ChimeraX.

Results and Discussion

Peptides with predicted antimicrobial and antibiofilm activities

According to the data obtained from the AMP Scanner, out of 13 proteins tested in Chia, 34 peptides showed antimicrobial activity above 0.5 probability, while 17 of them gave 0.9 or higher. 32 peptides were predicted to be antibiofilm active. Among the peptides derived from the 11 amaranth proteins, 12 peptides with antimicrobial and 22 with antibiofilm activity were predicted. Among the quinoa protein-derived peptides, 12 and 22 peptides were predicted to have antimicrobial and antibiofilm activities, respectively. Most AMPs are positively charged and support the interaction between the negatively charged lipid groups of the bacterial surface (Hadjicharalambous et al., 2022; Talapko et al., 2022) Complete data has been presented in Supplementary Table 1. Fig. 1 shows the histogram of the frequency of charges of the predicted antimicrobial peptides; a large majority falls in the range of 1 and 4. The cationic nature of antibiofilm peptides is also characteristic because of the multiple Arg and Lys residues that lead to electrostatic interactions with newly synthesized extracellular polymeric substances containing pyruvates and uronic acids (Uma Mahto et al., 2022). 65 peptides out of 76 peptides (which were predicted to have antibiofilm activity) bear positive charge ranging from 0.5 and 4.5. One of the mechanisms that AMPs exert on microorganisms prevent or destroy the biofilm formed in the early stage of colonization by degrading the membrane potential or the extracellular polymeric matrix, inhibiting the quorum sensing or alarmone systems (Yasir et al., 2018) Table 2 shows the overlapping predicted antimicrobial and antibiofilm activities of chia, amaranth and quinoa proteins after digestion with pepsin. The peptides of chia ADIRAAIPKHCWVKNPWKSMSYVVRDVAVVF, SDIKAAIPKHCWVKDPWRSVGYVVRDVVAVL, and TGVWVIAHECGHHAF can be considered promising antibacterial agents, but *in vitro* validations are still needed.

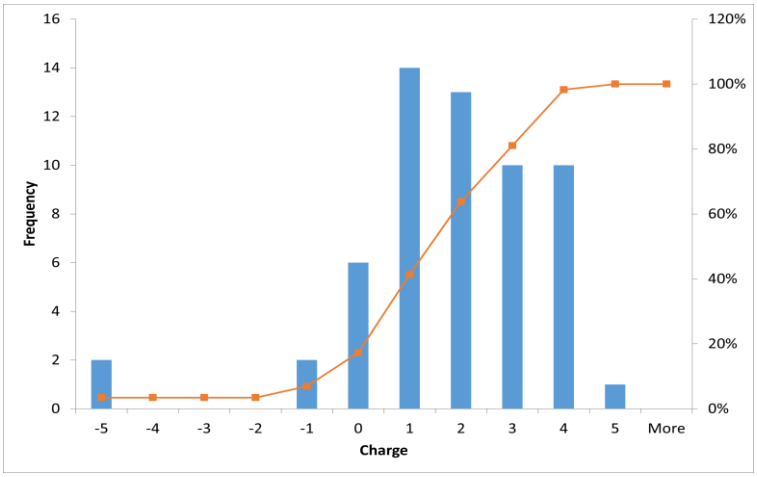

Figure 1. Histogram for the charge distribution of the predicted antimicrobial peptides.

Table 2. Predicted peptides with both antimicrobial and antibiofilm activity

Organism	Protein ID	Peptide sequence	Charge	pI	SVM score	AMP probability score
Chia	A0A1Z1EC60	NKIHHDIGTHVVHHL	2.50	7.47	1.01	0.82
	A0A1Z1EC53	NKIHHDIGTHVIHHL	2.50	7.47	0.72	0.73
	A0A1Z1EC64	ADIRAAIPKHCWVKNPWKSMSYVVRDVAVVF	3.50	9.64	1.07	0.94
	A0A1Z1EC52	SDIKAAIPKHCWVKDPWRSVGYVVRDVVAVL	2.50	9.17	1.03	0.91
	A0A1Z1EC55	TGVWVIAHECGHHAF	0.50	6.29	1.31	0.91
Amaranth	D6RSA4	YPEKARGVTKF	2.00	9.72	0.69	0.66
	A7LIU5	YKANRAHTYL	2.50	9.72	0.52	0.60
Quinoa	XP_021755284.1	AGKTSVWKAL	2.00	10.02	0.76	0.87

Table 3. Best docking energy scores of peptides with the target enzymes of *H. pylori*. The peptides with the highest docking scores for all three targets are labeled in bold.

Organism	Peptide sequence	Protein ID	D-alanine-D-alanine ligase	Type II dehydroquinase	Shikimate kinase
Amaranth	ESRKGKVKL	A7LIU5	-132.058	-139.963	-116.211
	NEQKKKF	A7LIU5	-121.258	-140.998	-124.226
	YKYRNPRQWL	A7LIU5	-195.066	-200.326	-143.304
	RHNKL	X4Y205	-150.276	-159.187	-148.533
	HPTKMAKSTNYF	Q38712	-197.709	-172.184	-154.327
	AAQQSPENPNWF	J9PE35	-174.161	-173.151	-141.113
Chia	QSRPHHL	A0A0F6PN28	-186.348	-184.875	-143.605
	SWKYSHRRHHSNTGSL	**A0A1Z1EC55 A0A1Z1EC46**	**-246.884**	**-206.374**	**-192.788**
	WSRSPGKKGSHF	A0A1Z1EC64	-175.915	-190.027	-169.765
	KAGVKEYKL	Q36769	-135.249	-143.424	-116.577
	RHNGL	XP_047958388	-159.381	-182.627	-136.556
	SPAHRSGKHF	XP_047974399	-173.871	-192.127	-142.078
Quino1a	TKRSKL	XP_021764389	-153.274	-139.707	-124.902
	EQGRGSSSQCRRQL	XP_021758596	-166.879	-154.535	-126.118
	MAKSTTTL	Q06AW2	-155.584	-141.014	-122.436

Peptides with predicted cell-penetrating activity and peptide-protein docking with the enzymes of H. pylori

116 peptides released from amaranth, chia, and quinoa proteins upon pepsin digestion were predicted to be cell-penetrating peptides (CPPs) (Table S1). From this group, 15 peptides with the highest probability of being CPPs and the highest uptake efficiency were used for peptide-protein docking with shikimate dehydrogenase, type II dehydroquinase, and D-alanine-D-alanine ligase. On the HPEPDOCK server, residues Arg57, Arg116, and Arg132 for shikimate dehydrogenase and residues Asn10, Leu11, Met13, Leu14, Arg17, and Tyr22 for type II dehydroquinase Leu308 and Tyr311 were specified prior to docking because of their involvement in catalysis (Wu et al., 2008; Paz et al., 2011; Chen et al., 2012). The top docking energy scores for each peptide for the target enzymes are listed in Table 3.

Of the 15 peptides subjected to peptide-protein docking, a peptide released from the chia seed proteins A0A1Z1EC55 and A0A1Z1EC46 with the sequence SWKYSHRRHHSNTGSL gave the highest docking energy score for all three enzymes selected as drug targets. Following this peptide, YKYRNPRQWL released from A7LIU5 of amaranth showed a high score for both D-alanine-D-alanine ligase and Type II dehydroquinase.

The 3D and 2D representations of SWKYSHRRHHSNTGSL docked to the target enzymes are shown in Fig. 2, and the non-bond interactions are presented in Table S2. D-alanine-D-alanine ligase interacts with the peptide to form conventional hydrogen bonds via Phe12, Glu13, Val19, Glu101, Ser177, and Tyr311, and carbon-hydrogen bonds between Ser177 and Arg8/Ser11, Asn310, and Trp2. Glu13 forms two hydrogen bonds with Arg8 at distances of 2.17839 Å and 2.80439 Å. Ser177 participates in a carbon-hydrogen bond with the Arg8 and Ser11 residues of the peptide at 3.07 and 2.82 Å, respectively.

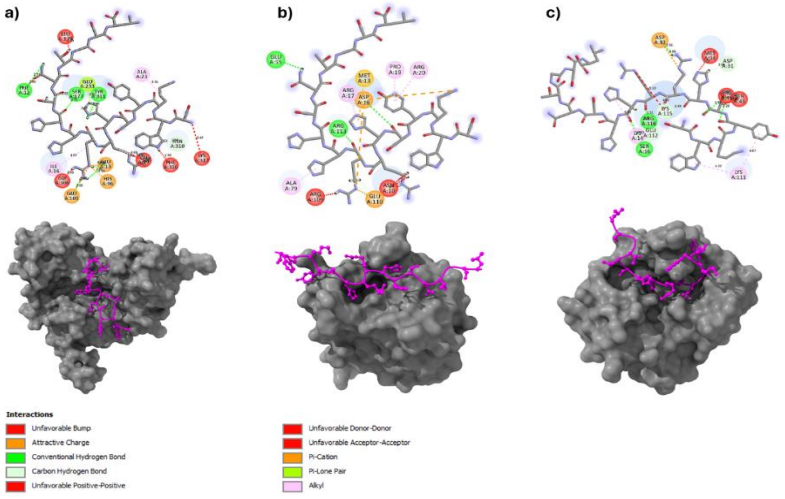

Figure 2. a) Interactions of SWKYSHRRHHSNTGSL with D-alanine-D-alanine ligase, b) type II dehydroquinase, c) and shikimate dehydrogenase.

Electrostatic interactions in the nature of attractive charge from Arg8 to Glu13 (4.63 Å) and Glu101 (5.58 Å) and π -cation from Arg8 to His96 were formed between the target and the peptide. In addition, hydrophobic and unfavorable types of interactions developed. For type II dehydroquinase, conventional hydrogen bonding occurred through residues Asn10, Asp18, Glu55, and Arg113 at distances of 2.27, 2.54, 2.61, and 2.16 Å, respectively. Glu110 of the target and Arg8 of the peptide interacted via a carbon-hydrogen bond at two sites at distances of 2.45 and 2.53 Å. Tyr4, His6, and His9 residues of the peptide form a π-alkyl-type hydrophobic interaction with Pro19, Arg20, Arg17, and Ala79 of the enzyme. Shikimate dehydrogenase-SWKYSHRRHHSNTGSL peptide docking analysis revealed that Ser16, Asp33, Arg45, and Arg116 of the enzyme participate in conventional hydrogen bonding. Arg116 is observed to form two hydrogen bonds with Arg8 at distances of 2.44 and 1.38 Å. His6 of the peptide interacts with the enzyme via carbon-hydrogen bonding with LYS115 and Asp31 at distances of 1.64 and 3 Å, respectively, while His9 forms the same type of interaction with Glu112 at 2 Å. Lys111 is involved in a pi-alkyl type of hydrophobic interaction with both Trp2 and Tyr4, whereas Lys14 of shikimate dehydrogenase and His9 of the peptide interact in the same way. Only one electrostatic interaction was observed for the type of attractive charge from Arg7 of the peptide to Asp33 of the target. Unfavorable interactions were also observed between the peptide and type II dehydroquinase or shikimate dehydrogenase. In view of the results of the docking analysis, His6, His9, and Arg8 were prominent residues of SWKYSHRRHHSNTGSL for peptide-protein interactions.

Conclusion

The aim of this study was to adopt an entirely new approach to the effect of ingested food on the early onset of *H. pylori* infection using in silico methods.1061 peptides with a length of 5 or more amino acids released by the peptic digestion of amaranth, chia, and quinoa proteins including recurring sequences due to the isoform proteins were analyzed. Predicted analysis revealed that chia seeds are a good source of antimicrobial and antibiofilm peptides. Moreover, a prominent peptide, SWKYSHRRHHSNTGSL, from the chia proteins A0A1Z1EC55 and A0A1Z1EC46 showed a high binding affinity for the selected enzymes of *H. pylori* compared to the other 14 peptides used for peptide-protein docking. Although the results of the *in silico* analysis were promising, especially for chia seeds, in vitro validation is required considering *in vivo* gastric conditions. Peptide sequences with high scores for the bioactivities investigated in this study can be tested for anti-Helicobacter activity, as well as other pathogens. To the best of our knowledge, this is the first work concerning the effect of ingested food *on H. pylori* infection and we believe that our results will provide valuable data and a new point of view for the scientists interested in this topic.

Ethical Statement

This work did not contain any animal or human studies performed by the authors.

Competing Interests

The authors have no competing financial interests to declare.

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This research received no external funding.

Data Availability

More data from this study are available in the supplementary files in the online version and also can be requested from the corresponding author.

Authors Contribution

E. Menfaatlı: Conceptualization, methodology, and writing - original draft; M. Kavruk: Visualization, writing - review, and editing; E. Gundeger: Supervision and methodology.

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