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In *Silico* Prediction of Bioactive Peptides from the Simulated Hydrolysis of a Wheat Gluten Protein

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Abstract: This study used bioinformatic tools to predict bioactive peptides derived from the high molecular weight DX5 subunit of wheat glutenin. Simulated hydrolysis with pepsin (pH > 2.0) was performed, and the resulting theoretical peptides were evaluated using PeptideRanker and ToxinPred 3.0 to assess bioactivity and toxicity. Among the generated fragments, ten showed a high probability of bioactivity (score ≥ 0.8), and four of them (IF, SF, WQ, and PG) were classified as non-toxic. These findings suggest their potential application in nutraceutical or therapeutic contexts. The in silico approach proves to be an effective and safe strategy for the preliminary identification of functional compounds, providing a foundation for further experimental validation.

Keywords: gluten, glutenin, bioactive peptides, in silico, prediction.

INTRODUCTION

Gluten is a complex mixture of storage proteins that share structural similarities but also exhibit distinct individual characteristics. The primary proteins in wheat gluten are known as gliadin and glutenin [1] and generally composed of 40–50% gliadin and 30–40% glutenin [2]. Gliadins are classified into four types: α, ß, γ, and ω; however, the α and ß groups are sometimes collectively referred to as α-type gliadins. On the other hand, glutenin polymers are high molecular weight compounds that, upon reduction of the disulfide bonds stabilizing their structure, can be separated into two subunits known as HMW (high molecular weight) and LMW (low molecular weight) subunits [3].

Gluten proteins are characterized by a high content of the amino acids glutamine and proline, ranging from 32–53% and 11–29%, respectively. Glutamine contains two nitrogen atoms, providing an adequate supply of this element to the seed during germination. Proline, due to its secondary amino group, contributes to the formation of a compact structure that facilitates storage in the endosperm, in addition to conferring resistance to most peptidases [4].

Peptides derived from gluten have been studied for their potential biological activities. One example is the opioid-like effect exhibited by some of these molecules. During digestion in the small intestine, gluten is hydrolyzed and releases peptides with structural similarities to endogenous opioid peptides, known as endorphins [5]. Additionally, antidiabetic peptides have been identified using a kiwi extract as a source of actinidin to hydrolyze gluten. Peptides with a molecular weight ≤1 kDa—particularly the WGLYH fraction—have shown inhibitory activity against α-amylase and α-glucosidase, two key

enzymes involved in glucose metabolism [6]. The above highlights that managing chronic diseases is a globally significant challenge, driving the search for safer, more sustainable therapeutic alternatives with fewer side effects. In this context, bioactive peptides derived from foods have gained considerable interest due to their multiple health benefits and promising potential in the treatment of these conditions [7].

In response to the limitations of *in vitro* and *in vivo* methods for identifying and producing bioactive peptides, bioinformatic tools have emerged as powerful alternatives. Leveraging peptide databases and specialized software, these *in silico* approaches enable more precise and efficient selection of proteins and enzymes as potential substrates for the release of specific bioactive peptides. Moreover, they support the prediction and analysis of occurrence frequency, molecular structure, potential toxicity, and biological mechanisms of therapeutic peptides using data available from specialized digital platforms [8]. There are various examples of the application of these tools in peptide research, such as the identification of peptides from *Nannochloropsis oculata*, where a research group conducted an *in silico* analysis that was crucial for predicting the bioactivity, stability, and potential therapeutic functions of the identified peptides, facilitating their selection prior to experimental validation. Using tools like PeptideRanker and specialized databases such as BIOPEP-UWM and PepBank, the inhibitory potential against ACE-1, antimicrobial activity, and the ability to act as pro-peptides after simulated gastrointestinal digestion were evaluated. These platforms enabled a more precise and efficient characterization, optimizing the design of functional peptides with nutritional and pharmacological applications [9].

Another example of the use of these tools is the analysis of a corn gluten hydrolysate, from which 449 peptides were identified. Using the PeptideRanker software, 20 peptides with scores above 0.75 were selected, excluding those with potential toxic or sensitizing effects. From these, 8 peptides (AWF, FAW, LWQ, WIY, YLW, LAYW, LPWG, and LYFY) were prioritized for synthesis, selecting those with a higher content of aromatic and hydrophobic amino acids due to their association with increased antioxidant activity [10].

The aim of this study is to generate and analyze theoretical peptides using *in silico* methods, starting from a characterized protein from wheat gluten. The goal is to identify fragments with potential bioactivity by evaluating their functional properties and toxicity profile, as a first step toward their possible application in the development of peptides for nutraceutical or therapeutic purposes.

MATERIALS AND METHODS

Amino Acid Sequence of High Molecular Weight Glutenin Subunit DX5 (GLT5_WHEAT) from *Triticum aestivum*

The sequence of the GLT5_WHEAT (**Table 1**) was obtained from UniProtKB [11]; its accession number is P10388 and it has a length of 848 amino acids.

Table 1: Sequence of the GLT5_WHEAT

10	**20**	**30**	**40**	**50**
MAKRLVLFVA	VVVALVALTV	AEGEASEQLQ	CERELQELQE	RELKACQQVM
60	**70**	**80**	**90**	**100**
DQQLRDISPE	CHPVVVSPVA	GQYEQQIVVP	PKGGSFYPGE	TTPPQQLQQR
110	**120**	**130**	**140**	**150**
IFWGIPALLK	RYYPSVTCPQ	QVSYYPGQAS	PQRPGQGQQP	GQGQQGYYPT
160	**170**	**180**	**190**	**200**
SPQQPGQWQQ	PEQGQPRYYP	TSPQQSGQLQ	QPAQGQQPGQ	GQQGQQPGQG
210	**220**	**230**	**240**	**250**
QPGYYPTSSQ	LQPGQLQQPA	QGQQGQQPGQ	AQQGQQPGQG	QQPGQGQQGQ
260	**270**	**280**	**290**	**300**
QPGQGQQPGQ	GQQGQQLGQG	QQGYYPTSLQ	QSGQGQPGYY	PTSLQQLGQG
310	**320**	**330**	**340**	**350**
QSGYYPTSPQ	QPGQGQQPGQ	LQQPAQGQQP	GQGQQGQQPG	QGQQGQQPGQ
360	**370**	**380**	**390**	**400**
GQQPGQGQPG	YYPTSPQQSG	QGQPGYYPTS	SQQPTQSQQP	GQGQQGQQVG
410	**420**	**430**	**440**	**450**
QGQQAQQPGQ	GQQPGQGQPG	YYPTSPQQSG	QGQPGYYLTS	PQQSGQGQQP
460	**470**	**480**	**490**	**500**
GQLQQSAQGQ	KGQQPGQGQQ	PGQGQQGQQP	GQGQQGQQPG	QGQPGYYPTS
510	**520**	**530**	**540**	**550**
PQQSGQGQQP	GQWQQPGQGQ	PGYYPTSPLQ	PGQGQPGYDP	TSPQQPGQGQ
560	**570**	**580**	**590**	**600**
QPGQLQQPAQ	GQQGQQLAQG	QQGQQPAQVQ	QGQRPAQGQQ	GQQPGQGQQG
610	**620**	**630**	**640**	**650**
QQLGQGQQGQ	QPGQGQQGQQ	PAQGQQGQQP	GQGQQGQQPG	QGQQGQQPGQ
660	**670**	**680**	**690**	**700**
GQQPGQGQPW	YYPTSPQESG	QGQQPGQWQQ	PGQGQPGYYL	TSPLQLGQGQ
710	**720**	**730**	**740**	**750**
QGYYPTSLQQ	PGQGQQPGQW	QQSGQGQHWY	YPTSPQLSGQ	GQRPGQWLQP
760	**770**	**780**	**790**	**800**
GQGQQGYYPT	SPQQPGQGQQ	LGQWLQPGQG	QQGYYPTSLQ	QTGQGQQSGQ
810	**820**	**830**	**840**	**848**
GQQGYYSSYH	VSVEHQAASL	KVAKAQQLAA	QLPAMCRLEG	GDALSASQ

In silico Hydrolysis using BIOPEP ENZYME(S) ACTION

In silico hydrolysis of GLT5_WHEAT was performed using the tool BIOPEP ENZYME(S) ACTION, the selected enzyme was pepsin at pH>2.0. Only sequences of two or more amino acids in length were retained, individual amino acids were discarded for bioactivity analysis [12].

Bioactivity Prediction using PeptideRanker

PeptideRanker assesses the potential bioactivity of peptides using an N-to-1 neural network model, considering peptides with a score greater than 0.5 as bioactive. This tool was applied to the peptides generated by *in silico* hydrolysis [13].

Toxicity Prediction using ToxinPred 3.0

ToxinPred 3.0 was employed to analyze the peptides generated through in silico hydrolysis. This enhanced version of the original platform utilizes machine learning and deep learning models trained on experimentally validated datasets of toxic and non-toxic peptides to classify each sequence as either "toxic" or "non-toxic" [14].

RESULTS

In silico Hydrolysis of the GLT5_WHEAT

The peptides generated *in silico* through simulated hydrolysis of the GLT5_WHEAT protein using the BIOPEP ENZYME(S) ACTION tool are presented in **Table 2**. Prior to bioactivity prediction using bioinformatics platforms, individual amino acids and duplicate fragments were removed to avoid redundancy (**Table 3**).

Table 2: Peptides generated *in silico*

M - A - K - RL - VL - F - VA - V - V - VA - L - VA - L - T - VA - E - G - E - A - SE - Q - L - Q - CE - RE - L - Q - E - L - Q - E - RE - L - K - A - CQ - Q - VM - D - Q - Q - L - RD - ISPE - CHP - V - V - VSP - VA - G - Q - Y - E - Q - Q - I - V - VPPK - G - G - SF - Y - PG - E - T - T - PPQ - Q - L - Q - Q - R - IF - WG - IPA - L - L - K - RY - Y - PS - VT - CPQ - Q - VSY - Y - PG - Q - A - SPQ - RPG - Q - G - Q - Q - PG - Q - G - Q - Q - G - Y - Y - PT - SPQ - Q - PG - Q - WQ - Q - PE - Q - G - Q - PRY - Y - PT - SPQ - Q - SG - Q - L - Q - Q - PA - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - PG - Y - Y - PT - SSQ - L - Q - PG - Q - L - Q - Q - PA - Q - G - Q - Q - G - Q - Q - PG - Q - A - Q - Q - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - L - G - Q - G - Q - Q - G - Y - Y - PT - SL - Q - Q - SG - Q - G - Q - PG - Y - Y - PT - SL - Q - Q - L - G - Q - G - Q - SG - Y - Y - PT - SPQ - Q - PG - Q - G - Q - Q - PG - Q - L - Q - Q - PA - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - PG - Y - Y - PT - SPQ - Q - SG - Q - G - Q - PG - Y - Y - PT - SSQ - Q - PT - Q - SQ - Q - PG - Q - G - Q - Q - G - Q - Q - VG - Q - G - Q - Q - A - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - PG - Y - Y - PT - SPQ - Q - SG - Q - G - Q - PG - Y - Y - L - T - SPQ - Q - SG - Q - G - Q - Q - PG - Q - L - Q - Q - SA - Q - G - Q - K - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - PG - Y - Y - PT - SPQ - Q - SG - Q - G - Q - Q - PG - Q - WQ - Q - PG - Q - G - Q - PG - Y - Y - PT - SPL - Q - PG - Q - G - Q - PG - Y - D - PT - SPQ - Q - PG - Q - G - Q - Q - PG - Q - L - Q - Q - PA - Q - G - Q - Q - G - Q - Q - L - A - Q - G - Q - Q - G - Q - Q - PA - Q - VQ - Q - G - Q - RPA - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - L - G - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PA - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - G - Q - Q - PG - Q - G - Q - Q - PG - Q - G - Q - PWY - Y - PT - SPQ - E - SG - Q - G - Q - Q - PG - Q - WQ - Q - PG - Q - G - Q - PG - Y - Y - L - T - SPL - Q - L - G - Q - G - Q - Q - G - Y - Y - PT - SL - Q - Q - PG - Q - G - Q - Q - PG - Q - WQ - Q - SG - Q - G - Q - HWY - Y - PT - SPQ - L - SG - Q - G - Q - RPG - Q - WL - Q - PG - Q - G - Q - Q - G - Y - Y - PT - SPQ - Q - PG - Q - G - Q - Q - L - G - Q - WL - Q - PG - Q - G - Q - Q - G - Y - Y - PT - SL - Q - Q -

T - G - Q - G - Q - Q - SG - Q - G - Q - Q - G - Y - Y - SSY - H - VS - VE - HQ - A - A - SL - K - VA - K - A - Q - Q - L - A - A - Q - L - PA - M - CRL - E - G - G - D - A - L - SA - SQ

Table 3: Selected peptides

RL - VL - VA - SE - CE - RE - CQ - VM - RD - ISPE - CHP - VSP - VPPK - SF - PG - PPQ - IF - WG - IPA - RY - PS - VT - CPQ - VSY - SPQ - RPG - PT - WQ - PE - PRY - SG - PA - SSQ - SL - SQ - VG - SA - SPL - VQ - RPA - PWY - HWY - WL - SSY - VS - VE - HQ - CRL

Predicted Bioactivity Probability of Peptides Generated *in silico* using PeptideRanker

In this platform, peptides with a score of 0.5 or higher are considered potentially bioactive; however, a threshold of 0.8 has been suggested to minimize the occurrence of false positives. **Table 4** displays the *in silico* generated peptides with scores ≥0.8.

Table 4: PeptideRanker scores

Peptide	Score
WG	0.992384
WL	0.987079
PWY	0.972403
IF	0.949173
SF	0.948796
WQ	0.909891
HWY	0.880483
PG	0.877086
CRL	0.832463
RPG	0.813194

Predicted Toxicity Probability *in silico* using ToxinPred 3.0

Toxicity prediction using ToxinPred 3.0 was conducted with the platform's default threshold (0.38) and model settings. It is important to note that this tool does not support the evaluation of single amino acids, but only peptide sequences consisting of two or more residues. Only peptides that scored 0.8 or higher in PeptideRanker were analyzed, to retain only non-toxic candidates (**Table 5**).

Table 5: ToxinPred 3.0 scores

Peptide	Score	Prediction
IF	0.334	Non-Toxin
SF	0.059	Non-Toxin

WQ	0	Non-Toxin
PG	0.314	Non-Toxin

DISCUSSION

The study demonstrated that the use of bioinformatics tools is an effective strategy for identifying peptides with potential biological activity from wheat glutenin, specifically from the high molecular weight subunit DX5. Through an *in silico* hydrolysis using pepsin at pH > 2.0 and subsequent analysis with specialized prediction platforms such as PeptideRanker and ToxinPred 3.0, ten peptides with a high probability of being bioactive (score ≥ 0.8) were selected, of which only four were classified as non-toxic.

The four selected peptides (IF, SF, WQ, and PG) had scores above 0.90 in PeptideRanker and were classified as non-toxic by ToxinPred 3.0, making them strong candidates for further * analysis. These findings are consistent with previous studies on peptides derived from corn gluten [10] or generated through simulated digestions with plant extracts [6], as some of the amino acids match in their sequences. The characteristics reported by those authors, such as hydrophobicity or aromatic structure, support the possibility that the sequences identified in our study may have functional value in the context of human health. On the other hand, while the *in silico* approach provides a fast, accessible, and risk-free pathway for the initial selection of promising compounds, it is crucial to validate these results through *in vitro* and *in vivo* assays to confirm their bioactive potential.

Overall, this work aligns with the growing trend toward the use of computational tools for the design and discovery of bioactive peptides from novel sources, contributing to the development of safe and sustainable alternatives for addressing diseases.

CONCLUSION

Using computational analysis, this study identified several peptides derived from the high molecular weight DX5 subunit of wheat glutenin, exhibiting bioactive potential with no apparent toxicity. Four peptides (IF, SF, WQ, and PG) received high scores in PeptideRanker and were classified as non-toxic by ToxinPred 3.0, positioning them as promising candidates for nutraceutical or therapeutic applications. These findings highlight the value of *in silico* approaches as a preliminary tool for the discovery of functional peptides and lay the groundwork for future experimental studies to validate their biological activity and safety profile.

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