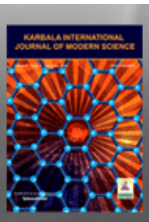
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Keywords

bread, CaATPase, HADDOCK, homology modeling, peptides

RESEARCH PAPER

Antifungal Peptides From Casein Milk of Etawa Crossbreed (*Capra hircus*) as Biopreservation Agent for Bread and Molecular Docking Studies

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Abstract

Bread without peptide fraction treatment started to grow mold on the fourth day with a total of 24 × 10^7 fungi colonies/ml. Meanwhile, the addition of peptide fractions GMC5, GMC6, GMC7, and GMC8 effectively preserved bread for up to 4 days, indicated by no mold growth. The best treatment was the GMC6 peptide fraction on day 8 which grew the least amount of fungus at 7 × 10^7 spores/ml. Peptide interaction with the CaATPase receptor *Aspergillus* sp. was carried out using HADDOCK 2.4. The 3D CaATPase structure prediction resulted in a model with good structural quality. This was supported by residues in favored regions at 99.53 %, a 3D-1D average score ≥0.2 with a percentage of 81.05 %, and a Molprobity score of 1.10. The four peptides exhibited great potential in inhibiting CaATPase through molecular binding with consecutive affinity energies of −6.7, −8.2, −7.7, and −5.6 kcal/mol for YNVPQLEIVPK, KENINELSK, GLSPEVPNENLLR, and YLGYLEQLLK, respectively. These peptides have the potential as bread preservatives.

Keywords: Bread, CaATPase, HADDOCK, Homology modeling, Peptides

1. Introduction

Antimicrobial peptides (AMP) are utilized as natural preservatives in bread, meat, and processed food products [1,2] due to their ability to prevent fungi contamination. The use of peptides as antifungals can preserve food without altering its quality or posing any harm [3]. According to a previous study, natural preservatives are increasingly used to improve food quality and safety [4]. Fungi, specifically the *Aspergillus sp.*, are common contaminants in foods and processed products. The peptide factions of the Goat Milk Casein (GMC), particularly 5, 6, 7, and 8, exhibited inhibitory effect against *Aspergillus* sp. Among them, GMC6 displayed the best activity with 26.95 ± 0.63 mm inhibition zone and 31.25 μg/ml a minimum inhibitory concentration (MIC). Four peptide sequence identified from GMC6 fraction of YNVPQLEIVPK, YLGYLEQLLK, KENNINELSK, and GLSPEVPNENLLR, exhibited ability to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria [5,6].

Several studies have explored the potential of other natural sources and peptides as preservatives. For instance, goat milk whey hydrolysate (HGW) has shown great efficacy in preserving bread for 2 days [7]. The peptide produced by *Lactobacillus plantarum* TE10 effectively inhibited the growth of *Aspergillus flavus* in maize seed [8]. Furthermore, peptides of Kenaf seed at concentrations of 1000 and 3000 mg/kg were able to prolong bread's shelf life by 10 and 16 days at a temperature of 25 °C [9]. Meanwhile, peptides extracted from palm kernel cake had the ability to prolong bread's shelf life for 10 days, especially at 2000 mg/kg concentration [10]. Homology modeling and molecular docking studies suggest that peptides

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such as EDQK, SEGGR, QDSIGS, DK, and EEK, attached to the T1R1, the umami taste receptor, with binding site at Arg151, Asp147, and Gln52 residues [11]. Several previous studies have been carried out on peptides from various sources as preservatives for food products and *in silico* testing of peptides for umami taste modulation. However, the use of peptides from goat milk protein as preservatives and their predicted interactions with CaATPase receptors have never been reported.

One prominent mechanism of action for antifungal peptide (AFP) is the inhibition of the CaATPase protein activity. Three synthetic peptides 66-10-FRLKFH, 77−3-FRLKFHF, and PPD1-FRLHF inhibit the growth of the fungus *Aspergillus flavus* and exhibit membranolytic activity. Docking analysis revealed that all peptides bind to proteins, particularly with high z-ranking score, when the peptides interact with the trans-membrane protein calcium ATPase from *A. Flavus* [12]. Furthermore, by consuming large amounts of ATP, plasma membrane ATPase regulates ion concentration to retain cell homeostasis and osmotic stability. A decrease in ATP ultimately leads to the suppression of ATPase [13]. The mechanism of this inhibition was accomplished through molecular docking.

Molecular docking is a computational chemical method that utilized to predict the affinity and binding interactions between ligands and target receptors [14]. CABS-dock, HPEPDOCK, and HADDOCK are several web servers available for protein-peptide docking. CABS-dock and HPEPDOCK conduct global docking by scanning the entire protein surface to determine the peptide binding site and mode [15]. Meanwhile, HADDOCK performs local docking [16], identifying binding poses around a user-defined site. The molecular interactions of peptides inhibiting proteins CaATPase on fungi cell membranes can be explored through molecular docking by employing the High Ambiguity Driven Docking (HADDOCK) web server. Generally, HADDOCK uses biochemical/biophysical experimental information translated into distance restraints, which guide docking toward conformations consistent with available experimental data [17]. Using HADDOCK 2.4 for molecular docking is easier and requires less time than AutoDock. Peptides as ligands interact more freely with receptor proteins, yielding docking results composed of clusters with each consisting of at least 8 peptide-protein complex models. HADDOCK scores and buried surface area were used for grouping to obtain maximum scores.

The 3D structure of CaATPase is not currently attainable in the Protein Data Bank (PDB), hence, the protein is constructed using homology modeling according to the sequence of the amino acid obtained from UniProtKB. Homology modeling is the preferred method for *in silico* construction of a three-dimensional protein structure in cases where a template is unavailable [18]. This approach is also widely used in virtual screening, mutagenesis experiment design, and analyzing the effects of sequence variation [19]. The computational process of homology-based methods is generally easier than fold recognition and ab initio techniques.

This study aims to test goat milk casein peptide fraction as a bread preservative, generate a CaATPase protein model through homology modeling, and subsequently dock the peptides with the CaATPase receptor to understand the interactions. The results are expected to provide a scientific reference in designing new compounds effective as antifungal agents, potentially for use as natural preservatives.

2. Materials and methods

2.1. Materials

This research utilized Etawa crossbred goat's milk from the experimental farm, Jenderal Soedirman University, Purwokerto, Indonesia, GMC5, GMC6, GMC7, and GMC8 peptides fraction; tween; Potato Dextrose Agar (PDA) (Merck); benzoic acid; flour; yeast; sugar; YNVPQLEIVPK, KENINELSK, GLSPEVPNENLLR, and YLGYLEQLLK peptides sequence; CaATPase amino acid sequence; as well as Lenovo Yoga core i7 laptop, CPU Core i7-12700KH, GPU NVIDIA RTX1650 4 GB. The internet connection enabled access to various online resources, including the UniProt server (http://www.uniprot.org), and the Robetta server (https://robetta.bakerlab.org/). Protein model Validation was confirmed at http://molprobity.biochem.duke.edu/, https://saves.mbi.ucla.edu/">https://swissmodel.expasy.org/assess, http://molprobity.biochem.duke.edu/, https://saves.mbi.ucla.edu/. Additionally, the prediction of protein active site residues was carried out using PrankWeb (https://prankweb.cz/), the HADDOCK web server (https://wenmr.science.uu.nl/HADDOCK2.4), and the Biovia Studio Discovery.

2.2. Fractionation of hydrolyzed protein

Casein protein hydrolyzed were fractionated using SPE SCX with buffer pH 3−9. The results of fractionation are peptides fraction was neutralized pH 7 and purified using a solid phase extraction polar enhance polymer (SPE-PEP) catridges with methanol as an eluent. Than, GMC pH X will be

called GMCX (X = pH value 3–9). The peptides fraction from the purification was dried by flowing nitrogen gas into the surface of the solution. Subsequently, the dried peptide fraction was dissolved in 1 mL of distilled water [5].

2.3. Bread preparation

The whole bread was prepared according to the method outlined by Ref. [3]. The ingredients include 480 g of wheat flour, 420 mL of water, 20 g of instant yeast, 5 g of salt, and 5 g of sugar. Wheat flour and salt mixed to taste were beaten in a separate bowl, added with bloomed yeast, and then mixed for 1.5 min to form a dough. Subsequently, a damp cloth was used to cover the dough, left the dough to rest in a warm place for 45 min, and then baked it for 35 min at 200 °C.

2.4. Determination of bread shelf life

Sliced bread was weighed up to 0.5 g, with the negative and positive control being bread without preservatives and the addition of benzoic acid, respectively. A bread was added with 50 μL GMC5, GMC6, GMC7, and GMC8 peptide fractions with 5000 μg/ml concentration. Each treatment was placed in a plastic clip and then incubated at 30 °C. Observations of fungi growth were carried out on days 1, 4, and 8 [10].

2.5. Calculation of the number of fungi spores

The growing colonies of *Aspergillus* sp fungi were counted using the TPC method on days 1, 4, and 8. The slices of bread were added to sterile water (100 mL) and subjected to intense stirring for 1 min to achieve uniform distribution. The mixture was serially diluted using sterile water up to 10^7. Subsequently, 50 μL of the suspension was taken and spread over PDA media. The suspension was incubated for 48 h at 30 °C to assess the fungal colony count [10].

2.6. Preparation of CaATPase protein sequences

The preparation of the CaATPase sequence was carried out through a comprehensive search in the UniProt database (http://www.uniprot.org).

2.7. Model building and validation of the CaATPase protein model structure

The CaATPase protein model was prepared using a homology modeling approach with the help of a web server (https://robetta.bakerlab.org/). This process began with submitting the amino acid sequence to the Robetta server. Consequently, 5 protein models were obtained, followed by evaluation employing the Swiss model (https://swissmodel.expasy.org/assess), Molprobity® (http://molprobity.biochem.duke.edu/), and Verify3D® (https://saves.mbi.ucla.edu/). Models having the best validation results were selected for molecular docking with peptides.

2.8. Peptide molecular attachment to CaATPase protein

The three-dimensional structure of the peptide was predicted using the USF Chimera 1.18 application. Molecular docking was conducted with the HADDOCK webserver (https://wenmr.science.uu.nl/HADDOCK2.4). In this process, peptide, CaATPase protein and amino acid residues on the active site of the receptor were inputted into the HADDOCK 2.4 webserver. CaATPase protein model and peptides were submitted as the first and second molecules, respectively. The docking protein-peptide was then selected, resulting protein-peptide complex model clusters. The three cluster models with the lowest HADDOCK scores were evaluated for molecular interactions using Biovia Studio Discovery. The binding affinity of peptide to receptor was determined by PRODIGY.

3. Results and discussion

3.1. Peptide fractions GMC5, GMC6, GMC7 and GMC8 as bread preservatives

The peptide fractions obtained were used as preservatives for white bread. Meanwhile, the negative control consisted of bread without preservatives, and the positive control included the addition of benzoic acid. About 0.5 g of bread was treated with the peptide fractions GMC5, GMC6, GMC7, and GMC8, particularly with 5000 μg/ml concentration. It was then undergoing incubation at 30 °C for 8 days with observation of growth on days 1, 4, and 8. Table 1 shows that the total mold on both the treated and the control white bread increased during storage. Bread without peptide fraction treatment started to grow mold on the fourth day, with the number of fungi colonies reaching 24×10^7 spores/ml. Meanwhile, GMC5, GMC6, GMC7, and GMC8 did not experience any fungal growth from day 1 to day 4, persisting until day 8. The GMC6 peptide fraction treatment on day 8 had the least amounts of fungi, at 7×10^7 spores/ml. Observations regarding bread treated with peptide fraction on days 1, 4, and

Table 1. Calculation of fungal colonies using the TPC method.

Peptide fraction	Number of fungi (spores/ml)		
	Day 1	Day 4	Day 8
Negative control	0	24×10^7	90×10^7
Positive control	0	0	3×10^7
GMC5	0	0	13×10^7
GMC6	0	0	7×10^7
GMC7	0	0	26×10^7
GMC8	0	0	17×10^7

8 are presented in Fig. 1. *Aspergillus* sp. fungi had grown on 1 white bread out of 3 samples (33.3 %) on day 3, with the other two samples only experiencing growth on day 4 at room temperature [20]. Bread has a relatively short shelf life without preservatives; therefore, chemical preservatives are often used by food manufacturers to enhance their stability and longer shelf life. As technological advancement occurs along with increased awareness of the consumers regarding the unfavorable consequences of chemical preservatives, natural sources of ingredients including peptides are proposed to replace commercial bread preservatives [21].

The GMC6 peptide fraction [5] can inhibit *Aspergillus sp.* by interacting with cell membranes. According to Ref. [12], the fungi samples that were treated with peptides showed no signs of sporulation, mainly attributable to the inhibition of the

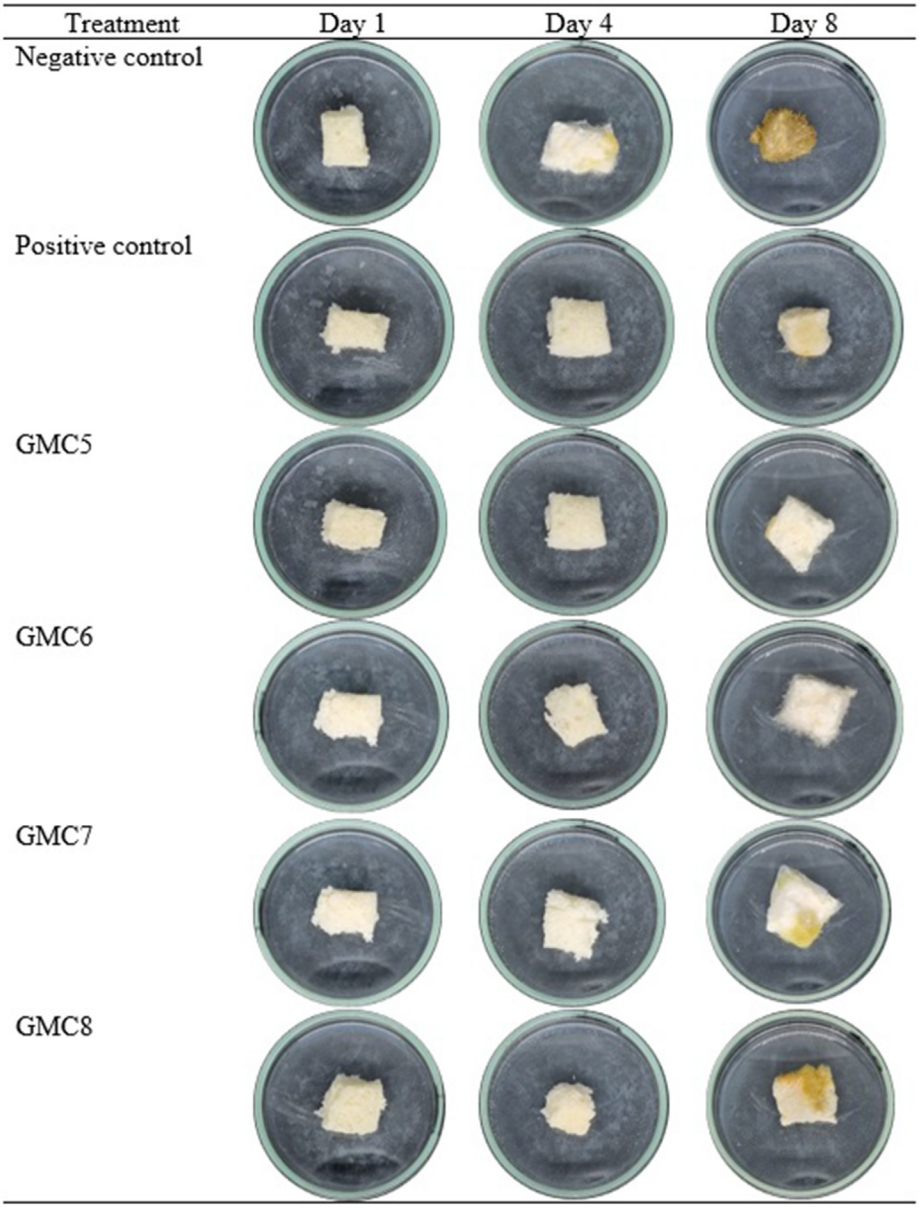

Fig. 1. Bread treated with peptide fractions on days 1, 4, and 8.

activity of calcium ATPase. This inhibition disrupts calcium homeostasis, which affects the sporulation process. The results demonstrate the potential of antifungal peptides produced as natural ingredients that are safer to use rather than synthetic agents for protecting bakery products from fungal growth.

3.2. Preparation sequences and model building of the ATPase receptor

Prior to docking, determination of receptors molecular structures must be carried out. The protein structures are generally retrieved from the website of the Protein Data Bank (PDB) [22]. CaATPase, a membrane transport located in the plasma membrane of *Aspergillus sp.*, lacks representation in the PDB. The amino acid sequence was downloaded from UniProtKB and subsequently used in the search and identification of templates applied in constructing the CaATPase model.

The Robetta web server was utilized to predict the 3D structure of the CaATPase protein based on ease of utilization and accuracy. Homology modeling has five stages, including the identification of templates, the alignment of target sequences, the creation of models for targets based on the 3D structure, refinement, and evaluation. Therefore, to obtain a 3D structure, homology modeling was carried out using the Robetta alignment server mode (https://robetta.bakerlab.org/) [23]. The server created a model based on the alignment of target amino acid sequences from UniProtKB with templates available in the PDB. Afterward, the protein was prepared by adding hydrogen atoms to increase the interaction accuracy, as crystallized protein structures often lost numerous hydrogen atoms. An evaluation and validation of the CaATPase protein model constructed was then performed. The amino acid sequence of CaATPase *Aspergillus sp.* is and the structure of the CaATPase protein are displayed in Fig. 2 and Fig. 3, respectively.

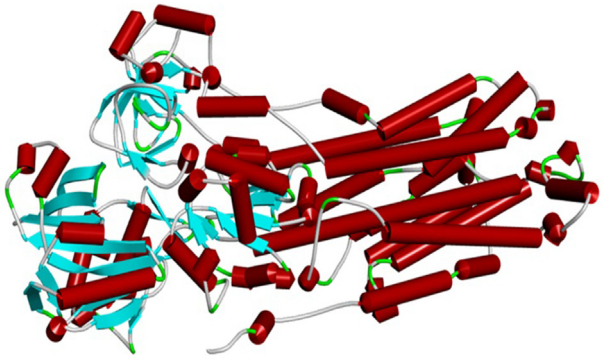

Fig. 3. Homology model of CaATPase receptor Aspergillus sp.

>tr|A0A3M2SXR5|A0A3M2SXR5_9EURO Calcium-transporting ATPase OS=Aspergillus sp. HF37 OX=1960876 GN=PHISP_06808 PE=3 SV=1MMENPALCSPKDALHHFSTSELSGLSQDQVLKARQEYGSNVLPEDPPTPLWHLV LEQFKDQLVIILLASAAISFVLALFEEGDDWTAFVDPAVILTILVLNAVVGVTQESNAEKAI AALQEYSANEATAVRDGTTQRVKADDLVPGDVIHVAVGDRIPADCRLLAIHSNSFRVDQ AILTGESESVAKDTRAIKDSQAVKQDQTNMLFSGTTVVNGHASAIVAFTGASTAIGDIHE SITSQISEPTPLKQKLNDFGDVLAKVITVICVLVWVINIEHFNDPSHGGWTKGAIYYLKIAV SLGVAAIPEGLAVVITTCLALGTRKMAQKNAVVRSLPSVETLGSCSVICSDKTGTLTTNQ MSVEKVAYLDKAGEGLEEIDVEGTTFAPEGKLSRDGKVLENLAVSSSTVRQMAEVMAL CNDANLSYDEKARAFSSIGEPTEGALRVLVEKIGADDAATNAELFRLPASKRLHAASSH YESRLPLNATYEFSRDRKSMSVLVGGEKEQKLLVKGAPESILERCSHVLLGPNGARVPV TKDHLDLLSTEVVEYGNRGLRVIALASVNDVGDNPLLHNASTTEEYAQLEQDLTLIGLVG MLDPPRVEVSDSIKKCRESGIRVIVITGDSPNTAEAICRQIGVFGEDENLVGKSFTGRQF DSLTEGEKVQAAKTASLFSRTEPSHKSMLVDILQSLGEVVAMTGDGVNDAPALKKSDIG VAMGTGTDVSKLAADMVLADDNFATITVAVEEGRSIYSNTQQFIRYLISSNIGEVVSIFLT AALGMPEALVPVQLLWVNLVTDGLPATALSFNPADHDVMRRPPRKRDESLVGGWLLF RYLVIGTYVGCATVFGYAWWFLYNPEGPQISLWELVGKRNIKSKENFYANFVQSHFHK CSAQFPEIGCEMFSNDMSKSASTVSLSILVVIEMFNAMNALSSSESLLSFGIWNNMMLV YAIVLSMALHFAILYVPFLRELFSILPLDLVEWKAVLAISAPVVVLDEILKFVERRLYAVPAV PIEQNGSASKPKRA

Fig. 2. The amino acid sequence of CaATPase receptor Aspergillus sp.

3.3. Validation of the ATPase receptor model structure

The assessment of the CaATPase protein structure on the Robetta server was presented through the Ramachandran Plots and MolProbity parameters. The results of the Ramachandran Plots model are depicted in Fig. 4.

Based on the Ramachandran Plots (a), proteins have very good structural quality when there are generally more amino acid residues in the favored region than the outliers. Table 2 shows that the CaATPase protein model had a favored area of 98.43 % and an outlier area of 0.29 %, implying a very good model. Additionally, it achieved 1.10 MolProbity score, further reflecting its relatively good quality. In addition, the MolProbity score incorporates a log-weighted integration of percentage of unfavorable Ramachandran, clash score, and bad side-chain rotamers. This score represents one expected value for describing crystallographic resolution. When the MolProbity score of the model structure observed to be lower than the actual crystallographic resolution, its quality is considered superior to the average structure. The crystallographic resolution of the template utilized for the CaATPase protein model was 1.15 Å [24]. The analysis results of the CaATPase Protein Model are exhibited in Table 2.

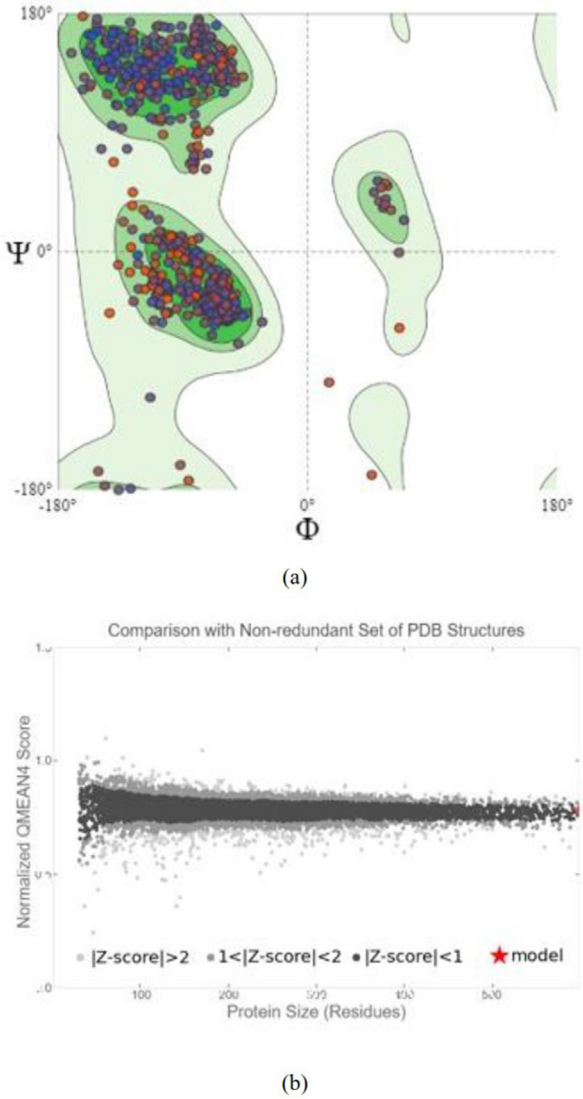

Fig. 4. (a) Ramachandran Plots of the CaATPase Protein Model, (b) Position of the Model (red star) in the distribution of Z values.

Table 2. The analysis results of the CaATPase protein model.

Parameter	Values	Targets (Goals)
MolProbity Score	1.10	
Clash Score	3.07	
Poor rotamers	0	<0.3 %
Favored rotamers	99.53 %	>98 %
Ramachandran Favoured	98.43 %	>98 %
Ramachandran Outliers	0.29 %	<0.05 %
C-Beta Deviations	0	0
Bad Bonds	0.1 %	0 %
Bad Angles	0.26 %	<0.1 %

Another parameter used to assess the quality of the model was QMEAN, and the website https://swissmodel.expasy.org/qmean/ was employed to find the QMEAN parameters. The CaATPase model obtained a score of 0.71. QMEAN is a composite scoring function that provides absolute quality estimates both globally (for the entire structure) and locally (for individual amino acid residue) grounded in a single model. The original QMEAN score ranged from 0 to 1, with 1 being regarded as good. By default, in order to establish a comparison with high-resolution X-ray structure, the values need to be converted to Z-scores. Fig. 4 illustrates the position of the model (red asterisk) on the distribution of Z values. The red asterisk remained in the Z-score <1 area, meaning that the model was relatively good. The quality of the protein model was assessed through the analysis of amino acid residues using 3D verification SAVESv6.0 [25]. The results of the current study revealed an average score of 3D-1D $\geq$ 0.2 at 81.05 %, suggesting good quality.

3.4. Docking of peptide molecules with CaATPase proteins

Peptides have been shown to possess promising potential as natural preservative candidates. This can be achieved through structural characterization of the underlying peptide-protein interactions, but the experimental characterization is quite difficult [26]. Furthermore, peptides vary in size, spanning

from a few residues to dozens of amino acids. To address this, bioinformatics and *in silico* methods offer a complementary approach for predicting the binding structure of protein-peptide complexes [27,28]. Molecular docking, which simulates optimal conformations based on complementarity and pre-organization, enables accurate prediction of interactions and binding affinity between ligands and receptors [29]. During docking, binding must comply with the mutual match theory, which involves complementary geometries, static electricity, hydrophobic interactions, and the bonds of hydrogen between the ligand and receptor [30].

The docking of CaATPase protein with peptides was performed by employing the HADDOCK 2.4 web server. In this study, molecular docking of the peptides was performed on the active site of the CaATPase protein's active site of Asp254, Asp257, Val258, Lys261, Val265, Val268, Ala303, Leu760, Ser763, Ser764, Glu767, Val768, Ile771, Leu824, Leu829, Tyr833, and Tyr837. The results of the binding interactions between CaATPase proteins and peptides are displayed by Table 3.

Table 3 shows that each peptide molecule, including YNVPQLEIVPK, KENINELSK, GLSPEVPNENLLR, and YLGYLEQLLK was subjected to docking with the CaATPase protein. The docking simulation yielded 10 clusters of CaATPase-peptide complex models, with the top three HADDOCK scores being selected. The peptides exhibited favorable interactions with the CaATPase protein, evidenced by binding affinity energies ranging from −5.1 to −8.3 kcal/mol. Docking of the carvacol (Thymus linearis) molecule with Plasma membrane ATPase produces a binding affinity energy of −5.4 using AutoDock vina [31]. Binding affinity energies of casein peptide with CaATPase are generally higher due to the difference in molecular types and docking methods. These negative values indicate that the resulting interactions are stable and spontaneous. *In silico* molecular docking studies aim to identify bioactive peptides that bind to the protein's active site that possess low binding affinities [32]. According to a previous study, compound with the best conformations possess the lowest energy levels [33]. The active binding site is a critical region on the macromolecule where ligands bind and potentially inhibit diseases [34]. Molecular docking has also been used to analyze the binding efficiencies of cyclic antifungal lipopeptides, including surfactin, iturin A, and fengycinto to the microtubular protein β-tubulin in plant pathogenic fungi [35]. Disruptions in calcium homeostasis can impact fungal sporulation and normal functions [36]. Antifungal compounds like tetrahydrocarbazole have been shown to hinder P-type ATPase activity [37].

3.5. Peptide-molecule interaction with CaATPase receptor

All clusters obtained from the molecular docking of peptides and CaATPase were examined to determine their intermolecular interactions using Biovia Studio Discovery. From each cluster, those with hydrogen bonds and the smallest binding affinity energies were selected, specifically clusters 2.3, 4.3, 4.1, and 1.1. The docking conformations of peptide and CaATPase protein were stabilized through intermolecular interactions, including hydrophobic, hydrogen bonding, and Van der Waals. The hydrophobic interactions identified in the results included pi-alkyl and alkyl interactions [38]. In addition, Hydrophobic and Van der Waals bonds also have a significant part in stabilizing ligand-receptor interactions. Smaller distances contributed to stronger hydrogen bonds, thereby stabilizing the protein-ligand binding. Furthermore, binding affinity was found to be influenced by intermolecular hydrogen bonds. The formation of hydrogen bonds is crucial for stabilizing the peptide interaction with

Table 3. Docking results of peptide molecules with CaATPase protein using HADDOCK 2.4.

Peptide	Models	HADDOCK score (au)	Van der Waals energy (kcal/mol)	Electrostatic energy (kcal/mol)	Buried surface area (Å^2)	Z-Score	ΔG (kkal/mol)
YNVPQLEIVPK	Cluster 1.2	−62.5	−37.1	−23.5	1292.3	−1.9	−6.5
	Cluster 2.3	−52.6	−31.1	−10.2	1210.8	−0.3	−6.7
	Cluster 6.2	−52.3	−32.1	−8.5	1218.5	−0.3	−7.0
KENINELSK	Cluster 4.3	−42.3	−30.7	−26.5	1115.6	−1.4	−8.2
	Cluster 3.3	−40.7	−31.5	−2.2	1007.6	−1.1	−8.3
	Cluster 1.1	−38.7	−30.4	−23.4	1051.9	−0.7	−8.1
GLSPEVPNENLLR	Cluster 4.1	−52.1	−36.0	−12.7	1214.9	−1.1	−7.7
	Cluster 2.3	−50.1	−36.8	−9.4	1268.7	−0.7	−7.9
	Cluster 3.2	−50.0	−36.7	−6.8	1173.6	−0.6	−7.7
YLGYLEQLLK	Cluster 3.4	−71.2	−37.8	−4.9	1217.3	−1.3	−5.1
	Cluster 1.1	−70.0	−37.6	−4.8	1201.9	−1.2	−5.6
	Cluster 4.4	−64.9	−35.0	−0.5	1100.6	−0.7	−5.5

the CaATPase receptor protein. Bond length denotes the mean distance between the nuclei of two atoms which are bonded in a molecule. Hydrogen bonds around the binding site can influence the strength of the compound binding to the receptor protein [39]. The hydrogen bond between the YNVPQLEIVPK peptide and the THR837 amino acid reached 2.04 Å in length. For the KENINELSK peptide, the observed bond lengths were 2.91 Å with the GLY826 amino acid, 2.59 Å with the THR 837 amino acid and 2.11 Å with the CYS841 amino acid. The GLSPEVPNENLLR peptide formed a hydrogen bond with the THR837 amino acid at a bond length of 2.99 Å. Furthermore, the YLGYLEQLLK peptides formed hydrogen bonds with LYS50 amino acid at 2.29 Å, LEU823 amino acid at 2.08 Å and VAL824 amino acid at 2.21 Å. Hydrogen bonding involves multiple hydrogen bond. Research by Ref. [40] shows that furfuryl alcohol has two hydrogen bonds with residues Gly-248 and Leu-270. The myosin heavy chain residues formed hydrogen bonds with *Staphylococcus carnosus* protease amino acid residues as follows: Phe 799 with Ser 43, Lys 205 with Gln 132, Ile 199 with Ala 133, Thr 141 with Gln 130, and Gln 806 with Ser 22 [41]. The resulting hydrogen bond formed has a length of <3.00 Å. According to Ref. [42], hydrogen bonds are considered strong when their a bond length is less than 3.00 Å. Hydrogen bond plays a significant part in stabilizing the interaction between the peptide and the CaATPase receptor protein. Peptides interact by hydrogen bonding in P4-ATPase with charged residues [43]. The peptide interactions with the CaATPase protein are shown in Table 4 and Fig. 5.

Table 4. Results of CaATPase peptide and protein interactions.

Peptides	Models	Hydrogen Bond	Van der Walls	Hydrophobic Interaction
YNVPQLEIVPK	Cluster 2.3	THR837	LYS868, ILE272, ILE264, PHE771, VAL767, VAL768, VAL257, LYS260	LEU268, LEU829, LEU833, ILE265, VAL261, VAL264, ALA775
KENINELSK	Cluster 4.3	GLY826, THR837, CYS841	PHE830, VAL261, ILE764, VAL767, ILE272	VAL264, LEU268, ILE265, PHE771, VAL768, LEU772, LEU833, LEU829
GLSPEVPNENLLR	Cluster 4.1	THR837	LEU776, ALA775, VAL767, ILE764, VAL264, PHE254, LEU258, CYS841	LEU772, VAL768, LEU268, ILE265, LEU833, LEU829, VAL261
YLGYLEQLLK	Cluster 1.1	LYS250, LEU823, VAL824	LEU251, VAL257, LEU829, LEU833, PHE830, LEU268, ILE764, LEU258, GLY825, PHE254, SER822	LYS260, VAL261, ILE265, VAL264, ASP253

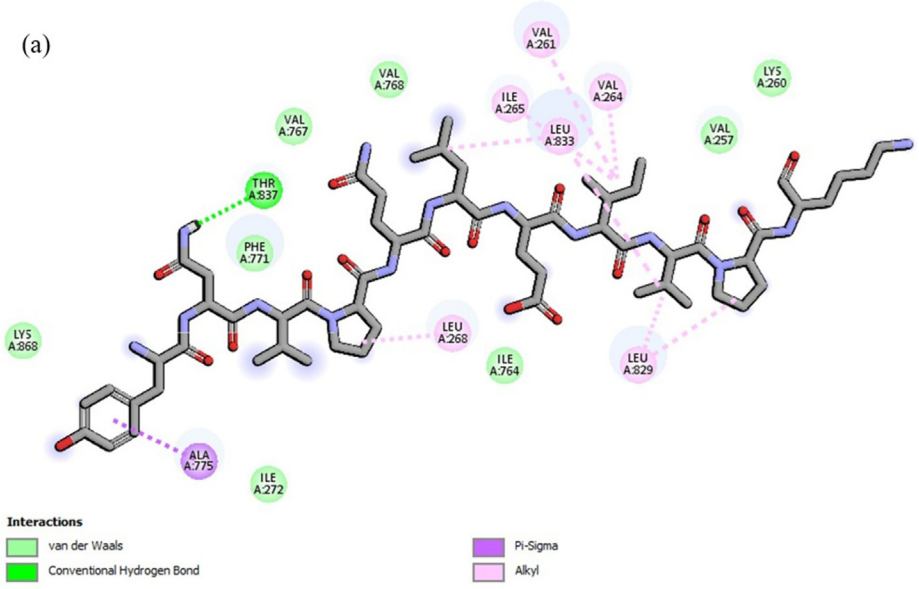

Fig. 5. Interaction of peptide compounds (a) YNVPQLEIVPK and (b) KENINELSK (c) GLSPEVPNENLLR and (d) YLGYLEQLLK on the CaATPase protein.

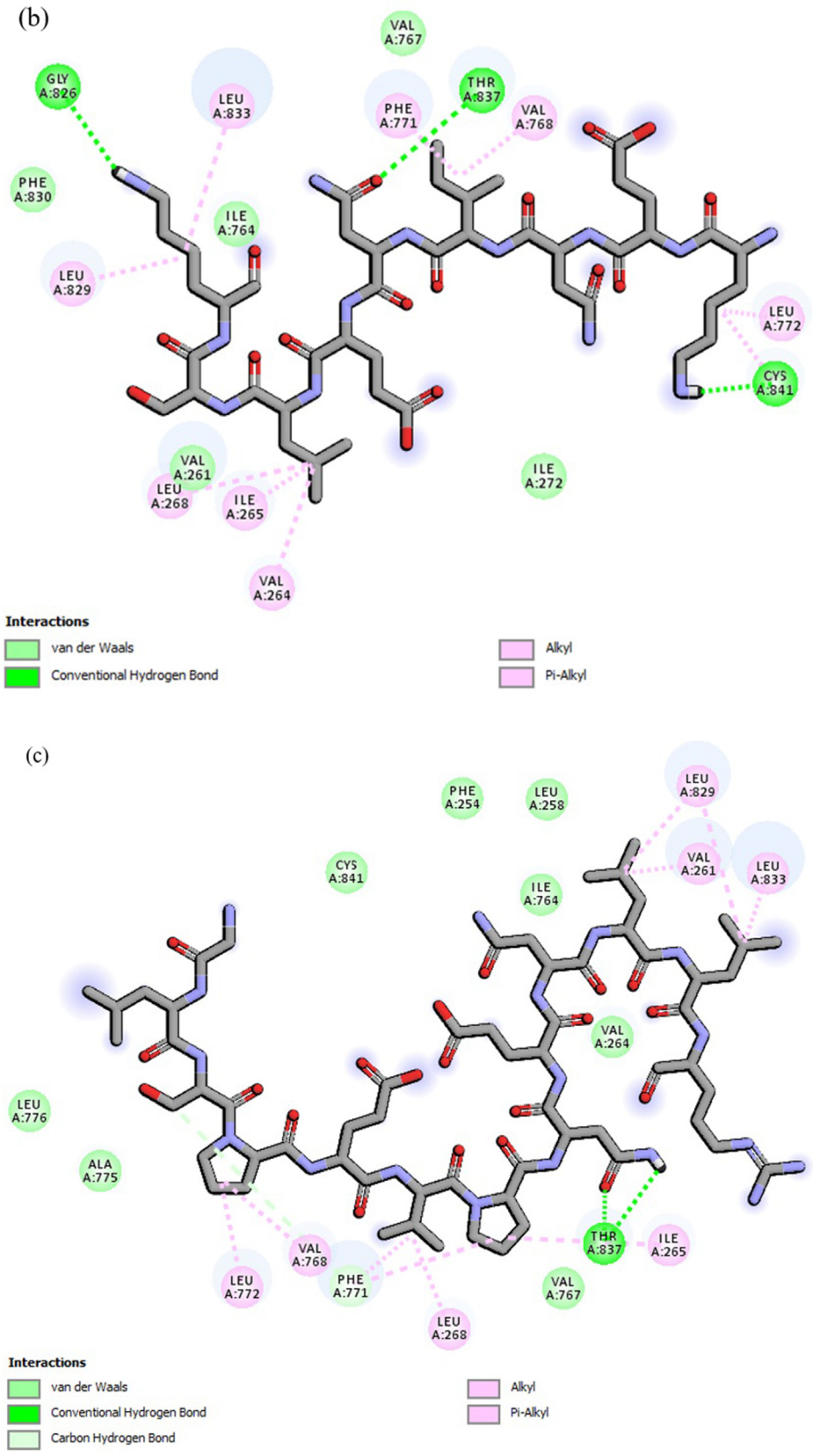

Fig. 5. (Continued).

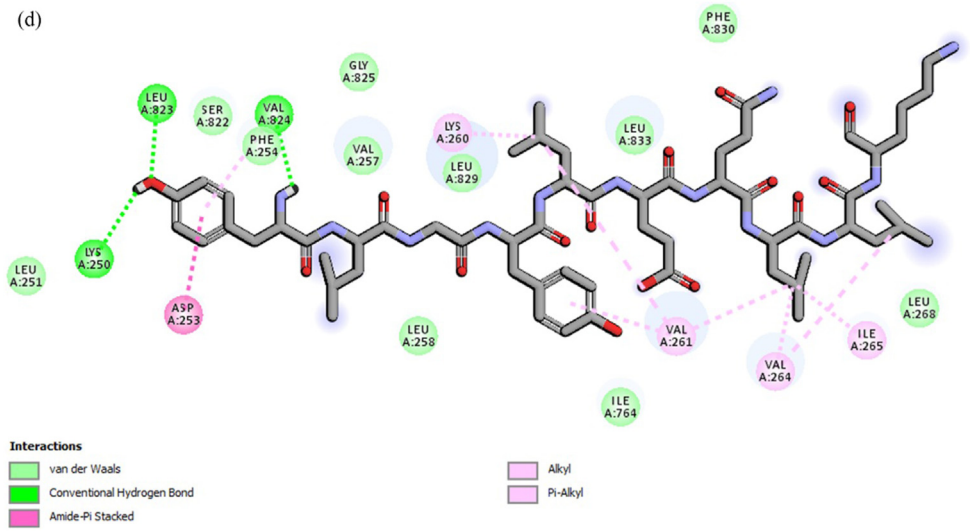

Fig. 5. (Continued).

4. Conclusions

In conclusion, the addition of peptide fractions GMC5, GMC6, GMC7, and GMC8 could preserve bread for up to 4 days, indicated by no mold growth. The best treatment was the GMC6 peptide fraction on day 8 which had the least amounts of fungi, reaching 7×10^7 spores/ml. The predicted 3D structures of the CaATPase receptor using the Robertta server produced protein models with good structural quality. This was supported by residues in favored regions at 99.53 %, a 3D-1D average score $\geq$0.2 with a percentage of 81.05 %, and a Molprobity score of 1.10. Peptides YNVPQLEIVPK, KENINELSK, GLSPEVPNENLLR, and YLGYLEQLLK exhibited the potential to inhibit CaATPase with successive binding affinity energies of −6.7, −8.2, −7.7, and −5.6 kcal/mol, respectively. Compounds with the best conformations tended to have low energy. These peptides have the potential to serve as bread preservatives.

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Ethics information

None.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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