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Molecular docking studies on α-amylase inhibitory peptides from milk of different farm animals

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ABSTRACT

Milk-derived peptides have emerged as a popular mean to manage various lifestyle disorders such as diabetes. Fermentation is being explored as one of the faster and efficient way of producing peptides with antidiabetic potential. Therefore, in this study, an attempt was made to comparatively investigate the pancreatic α-amylase (PAA) inhibitory properties of peptides derived from milk of different farm animals through probiotic fermentation. Peptide's identification was carried out using liquid chromatography-quadrupole time-of-flight mass spectrometry and inhibition mechanisms were characterized by molecular docking. Results obtained showed a PAA-IC_{50} value (the amount of protein equivalent needed to inhibit 50% of enzymes) between 2.39 and 36.1 μg protein equivalent for different fermented samples. Overall, *Pediococcus pentosaceus* MF000957-derived fermented milk from all animals indicated higher PAA inhibition than other probiotic derived fermented milk (PAA-IC_{50} values of 6.01, 3.53, 15.6, and 10.8 μg protein equivalent for bovine, camel, goat, and sheep fermented milk). Further, molecular docking analysis indicated that camel milk-derived peptide IMEQQQTEDEQQDK and goat milk-derived peptide DQHQKAMKPWTQPK were the most potent PAA inhibitory peptides. Overall, the study concluded that fermentation derived peptides may prove useful in for managing diabetes via inhibition of carbohydrate digesting enzyme PAA.

Key words: milk fermentation, probiotics, α-amylase inhibition, antidiabetic bioactive peptides, molecular docking

INTRODUCTION

Milk and milk-derived products not only have a substantial role in the human nutrition, but they are also considered a rich source of essential nutrients required by humans. These products serve as excellent sources of proteins, lipids minerals, and vitamins (Silva et al., 2020). Milk can be fermented by different microbes, *Lactobacillus* spp. or bifidobacteria, to produce a partially digested milk with varying properties (Salli et al., 2021). Upon fermentation of milk, lactose sugar and proteins are broken down into simpler sugar (glucose and galactose) and peptides, respectively. Moreover, the breakdown of proteins could result in bioactive peptides with diverse pharmaceutical and nutraceutical potentials, for example antioxidant, antidiabetic, antiobesity, anti-inflammatory, anticancer, and antihypertension activities (Linares et al., 2017; Marco et al., 2017; Mathur et al., 2020).

Antidiabetic peptides can reduce level of blood glucoses, improve insulin uptake, and restrain some important enzymes associated with carbohydrate metabolism and glucose absorption (Antony and Vijayan, 2021). Peptides with antidiabetic activities have been mainly screened via their capability to inhibit major carbohydrases (pancreatic α-amylase [**PAA**] and α-glucosidase [**AG**]), and dipeptidyl peptidase IV (**DPP-IV**) an enzyme implicated in degradation of insulinotropic hormones. The PAA inhibition can reduce the enzyme activity and thus decrease the digestion rate of starch and disaccharides into glucose, causing a slow secretion and reduced absorption of glucose in the gut (Karimi et al., 2020). Previous studies have shown the enhanced efficacy of fermented dairy products toward PAA inhibition in comparison with their unfermented form. This is largely attributed to release of bioactive peptides from intact proteins via bacterial proteases during fermentation. For example, cow and

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camel milk-based yogurt fermented by *Allium sativum* showed higher PAA inhibitory activity in comparison with unfermented samples (Shori and Baba, 2014). In addition, fermentation of soya milk by kefir has been reported to produce bioactive peptides with significant PAA inhibitory potential (Tiss et al., 2020). In fact, camel milk fermented with probiotic *Lactobacillus* spp. has shown significantly higher PAA inhibitory capability in comparison to unfermented milk (Ayyash et al., 2018b). Moreover, significant differences between the PAA inhibitory potential of camel and cow milk were reported as well.

The milk composition of different mammals differs significantly. The type of species, genes, physiological, nutritional, and environmental factors influence the composition of mammal milk and thus their protein content and composition (Pietrzak-Fiecko and Kamelska-Sadowska, 2020). Differences in protein composition and content between different mammals will result in the production of a wide range of peptides upon milk fermentation. Those peptides will vary in composition, sequence, molecular weight, and bioactivity. In fact, microbial fermentation offers uncontested advantages over other methods of peptides production due to their broad range of microbial proteases can produce peptides of varying sizes and sequences that may result in superior biological activity and their characteristics (Khan et al., 2018).

To the best of our knowledge, previous literature related to obtaining bioactive peptides with PAA inhibitory activities via fermentation from major farm animals has been quite limited. Only a few studies that have been conducted are majorly carried out on comparison between bovine and camel milk. However, looking at the increasing demand for alternative milk sources such as goat and sheep milk, a thorough investigation in this direction is necessitated. Therefore, in this study, bovine, camel, goat, and sheep milk were fermented by *Lactiplantibacillus argentoratensis* MF000943 (**LPA**), *Limosilactobacillus fermentum* MF000944 (**LiF**), *Lactiplantibacillus pentosus* MF000946 (**LPe**), *Pediococcus pentosaceus* MF000957 (**PPe**), and *Enterococcus hirae* MF000958 (**EH**). The degree of proteolysis and PAA inhibitory activity of fermented milk was measured on d 0, 7, and 14 of refrigerated storage. Finally, the peptides present in PPe fermented milk samples were identified and sequenced for in silico PAA inhibitory activity.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Materials

Fresh raw milk samples from same breed animals (i.e., bovine, Holstein Friesian) camel (Omani), goat (Salali) and sheep (Al-Nuaimi breed; n = 3 for each milk type), were obtained under refrigerated conditions from local farms of Al Ain, an eastern region of Abu Dhabi Emirates, United Arab Emirates. Various probiotic microorganisms used in this study for fermentation were isolated from camel milk. The probiotics obtained were identified using 16s rRNA sequencing and upon submission to the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/genbank/) the accession number was obtained. The following probiotics were used: LPA, LiF, LPe, PPe, and EH. The PAA from porcine pancreas, p-nitrophenyl-α-D-maltohexaoside as amylase chromogenic substrate, were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals used in this study were of analytical grade.

Protein Hydrolysis Via Microbial Fermentation

Fresh milk samples obtained above were skimmed 2 times by refrigerated centrifugation at 4,000 × *g* and 10,000 × *g* for 15 min each at 4°C. These skim milk samples were then heated at 90°C for 10 min causing whey protein denaturation and then cooled down to 37°C in ice-cold water. Probiotic inoculation was performed at a level of 10^5 cfu/mL from 18-h-old cultures in triplicate, and inoculated milk samples were fermented under static conditions for 24 h at 37°C. Following fermentation, fermented milk samples were stored at 4°C for 2 weeks and a weekly analysis was performed until 14 d. Briefly, at each sampling interval, fermented milk samples were first neutralized to pH 7.0 using 1 *M* NaOH and clear water-soluble extracts (**WSE**) containing peptides were obtained via centrifugation at 10,000 × *g* for 15 min at 4°C.

Determination of Degree of Hydrolysis

Based on the original method of (Nielsen et al., 2001), the degree of hydrolysis (**DH**) was determined by o-phthaldialdehyde method as further modified by (Mudgil et al., 2019):

$$DH\,(\%) = \left(\frac{h}{h_{tot}}\right) \times 100,$$

where h and h_{tot} represent the amount of hydrolyzed bonds and total amount of peptide bonds per protein equivalent, respectively. The amount of hydrolyzed bonds was estimated using $h = (\text{SerineNH}_2 - \beta)/\alpha$, where α, β, and h_{tot} values were taken from (Nielsen

et al., 2001) as 1.039, 0.383, and 8.2 mEq/g of protein, respectively.

PAA Inhibition Assay

The PAA inhibitory activity potential was measured as per the method of (Baba et al., 2021b). Briefly, various concentrations of WSE from fermented milk were incubated with 100 μL of PAA enzyme (2 mg/mL) in sodium phosphate buffer (0.02 *M* with 0.006 *M* sodium chloride; pH 6.9), and preincubation at 37°C was carried out for 15 min. Thereafter, 50 μL of substrate p-nitrophenyl-α-maltohexaoside (5 m*M*) was added for initiating the reaction and incubation was carried out at 37°C for 60 min. The absorbance of p-nitrophenyl developed was monitored at 405 nm using a microplate reader (Epoch 2, BioTek, Winooski, VT). For each test reaction their reaction blanks with sample and buffer were run to minimize background noise. The percent inhibition of enzyme activity was calculated at each concentration of sample using the following equation:

$$\%\text{AAL inhibition} = \frac{\text{Abs}_{Control} - \text{Abs}_{Test\ Sample}}{\text{Abs}_{Control}} \times 100.$$

The term $\text{Abs}_{Control}$ is the absorbance of the control sample with enzyme and substrate but without the test sample, whereas $\text{Abs}_{Test\ Sample}$ is the absorbance of the test reaction with enzyme, substrate, and test samples at different concentrations. The amount of protein equivalent needed to inhibit 50% of enzymes is IC_{50}. The inhibition values were plotted against the concentration of WSE as micrograms of protein equivalent, and IC_{50} values were determined from the slope of the curve.

Peptide Identification

Fermented milk samples from PPe were selected for peptide identification using liquid chromatography-quadrupole time-of-flight MS as previously described by (Sarah et al., 2016) and (Mudgil et al., 2022). The detailed method is described in Supplemental File S1 (https://data.mendeley.com/datasets/z2nd7c4p33/1, Mudgil et al., 2024). The identified peptides were first subjected to novelty check through databases such as BIOPEP-UWM (https://biochemia.uwm.edu.pl/); PepBank (http://pepbank.mgh.harvard.edu/); PeptideDB (http://www.peptides.be/); and EROP-Moscow (http://erop.inbi.ras.ru/). Blind peptide protein docking using HPEPDOCK server (http://huanglab.phys.hust.edu.cn/hpepdock/) was performed for screening peptides interaction with PAA (Zhou et al., 2018).

Molecular Binding Mechanism of Identified Peptides Against PAA

Molecular interactions of identified peptides with PAA were performed to authenticate the mechanism of PAA inhibition. For this, Crystal Structure of Human Salivary Enzyme (PDB; 1SMD) was downloaded from the RCSB Protein Data Bank database (https://www.rcsb.org/structure/1smd), and the pepsite 2 (http://pepsite2.russelllab.org/) web server was used for understanding molecular binding of peptides with PAA (Trabuco et al., 2012). The most potent PAA inhibitory bioactive peptide was selected based on the significance of binding ($P < 0.05$) and total number of potential binding sites on target enzyme PAA.

SAR Analysis

The 3-dimensional structure–activity relationship (**SAR**) of PAA (PDB; 1SMD) from RCSB Protein Data Bank (http://www.rcsb.org) was downloaded and peptide structure was generated using the PepFold 3 server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/#overview). Consequently, the peptide docking into the enzyme structure was completed using the High Ambiguity Driven protein-protein DOCKing (HADDOCK) server (http://wenmr.science.un.nl/haddock2.4/) as per the methodology described by (Honorato et al., 2021). Thereafter, PAA interactions with selected peptides were investigated using docking analysis.

Statistical Analysis

All the experiments were carried out in triplicate and the data analysis was performed via one-way ANOVA using SPSS version 28.0 software (SPSS Inc., Chicago, IL). Mean significant differences among different fermented samples were separated using Tukey's new multiple range test for establishing significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Degree of Hydrolysis

Extent of proteolysis from fermented milk samples obtained by different probiotics was determined on d 0, 7, and 14 of refrigerated storage and results obtained are shown in Table 1. The unfermented control had the lowest DH% value and varied significantly ($P \leq 0.05$) throughout the study. This inherent DH in unfermented control milk sample on d 0 and during the storage suggests that proteases from native microbial

flora might have caused some degradation of proteins. The DH% of different probiotic fermented bovine milk on d 0 was similar, without any significant differences, apart from the LPA fermented sample that had the lowest DH% (25.95% ± 0.13%). By d 7, the DH% of the samples ranged between 37 and 43%. By d 14, LPe fermented bovine milk showed the highest DH% (57.06% ± 1.34%), being significantly higher than the other probiotic fermented bovine milk ($P \leq 0.05$). In fact, this DH% value was significantly higher compared with LPe fermented milk from other animals by d 14.

Initiation of camel milk fermentation on d 0 resulted in significantly varying DH% between the samples ($P \leq 0.05$). The LPe fermented camel milk sample had the highest DH% on d 0 (33.73% ± 0.82%) compared with the other camel milk samples. Different fermented camel milk samples showed similar DH% by d 7, however by d 14, LiF showed the highest DH% value (47.80% ± 1.39%) significantly different than the other camel milk samples ($P \leq 0.05$). The DH% values of other milk sources fermented by LiF were similar on d 14 with no significant difference ($P > 0.05$). A study by (Moslehishad et al., 2013b) reported that *Lactobacillus fermentum* PTCC 1638 and *Lactobacillus rhamnosus* PTCC 1637 showed high protease activity in fermenting camel and cow's milk.

Goat milk samples had the highest DH% values since d 0, this indicates the capability of goat proteins to undergo fermentation and production of peptides at a faster rate compared with the milk of other animals. This is also clear when the DH% values of goat milk samples of d 7 and 14 are compared with the other animals. Goat milk samples always had the highest DH% values with 2 significant exceptions on d 14 for LPA and LPe. The EH produced the significantly higher number of peptides as indicated by degree of hydrolysis on d 7 (53.85% ± 0.46%) and d 14 (55.62% ± 0.33%) in comparison to the other fermented goat milk samples ($P \leq 0.05$). In addition, this DH% value was significantly higher compared with other milk sources fermented by EH by d 14.

Among the fermented sheep milk samples of d 0, an unexpected DH% value was determined for LPe fermented sheep milk (14.96% ± 1.21%). This was the lowest DH% value among all the other fermented sheep milk samples and the other animals' fermented milk of d 0, the difference was significant ($P \leq 0.05$). By d 7, sheep milk fermented by LiF resulted in the highest value of DH% (42.58% ± 0.79%) which was significantly different that the other samples of that day and similar to that of goat milk on the same day. In fact, on d 14, *L. fermentum*-fermented sheep milk also had the highest DH% value (44.67% ± 0.33%) with a significant difference in comparison to the other fermented sheep milk on d 14. This value is significantly higher than the *L. fermentum*-fermented camel milk of d 14,

Table 1. Degree of protein hydrolysis of fermented milk from different farm animals upon fermentation by different probiotic microorganisms[1]

Storage period (d)	Organism[2]	Degree of hydrolysis (%)			
		Bovine	Camel	Goat	Sheep
0	UC	$3.678 \pm 0.48^{a,A}$	$2.649 \pm 0.16^{a,A}$	$3.065 \pm 0.54^{a,A}$	$3.019 \pm 0.47^{a,A}$
	LPA	$25.95 \pm 0.13^{b,A}$	$24.91 \pm 0.76^{b,A}$	$39.37 \pm 0.61^{bc,C}$	$30.42 \pm 1.50^{d,B}$
	LiF	$28.01 \pm 0.48^{c,A}$	$31.07 \pm 0.72^{d,A}$	$44.28 \pm 9.24^{c,B}$	$30.59 \pm 1.82^{d,A}$
	LPe	$27.93 \pm 0.43^{c,B}$	$33.73 \pm 0.82^{e,C}$	$45.86 \pm 0.76^{c,D}$	$14.96 \pm 1.21^{b,A}$
	PPe	$27.94 \pm 0.64^{c,AB}$	$28.85 \pm 0.13^{c,B}$	$31.41 \pm 0.70^{b,C}$	$26.88 \pm 0.54^{c,A}$
	EH	$28.03 \pm 0.39^{c,B}$	$25.12 \pm 1.29^{b,A}$	$43.93 \pm 0.11^{c,C}$	$26.35 \pm 1.17^{c,AB}$
7	UC	$5.044 \pm 0.64^{a,A}$	$5.253 \pm 0.37^{a,A}$	$5.849 \pm 0.79^{a,A}$	$5.358 \pm 0.74^{a,A}$
	LPA	$38.29 \pm 1.50^{bc,B}$	$35.03 \pm 0.87^{bc,A}$	$43.84 \pm 0.54^{b,C}$	$42.58 \pm 0.79^{c,C}$
	LiF	$37.07 \pm 1.20^{b,AB}$	$35.46 \pm 1.07^{bc,A}$	$45.76 \pm 0.21^{c,C}$	$38.62 \pm 1.11^{b,B}$
	LPe	$42.99 \pm 0.55^{d,C}$	$33.36 \pm 1.18^{b,A}$	$52.21 \pm 0.60^{d,D}$	$37.95 \pm 0.36^{b,B}$
	PPe	$37.36 \pm 0.90^{b,B}$	$33.71 \pm 0.69^{b,A}$	$44.09 \pm 0.36^{b,C}$	$38.59 \pm 0.33^{b,B}$
	EH	$40.42 \pm 0.68^{cd,B}$	$37.17 \pm 1.03^{c,A}$	$53.85 \pm 0.46^{e,C}$	$37.29 \pm 0.00^{b,A}$
14	UC	$8.094 \pm 0.09^{a,B}$	$6.023 \pm 0.07^{a,A}$	$6.306 \pm 0.55^{a,A}$	$7.605 \pm 0.31^{a,B}$
	LPA	$49.81 \pm 0.54^{b,D}$	$41.41 \pm 0.85^{c,A}$	$47.37 \pm 0.02^{b,C}$	$44.67 \pm 0.33^{b,B}$
	LiF	$48.97 \pm 0.11^{b,B}$	$47.80 \pm 1.39^{d,B}$	$48.07 \pm 0.20^{b,B}$	$39.49 \pm 0.34^{b,A}$
	LPe	$57.06 \pm 1.34^{c,D}$	$43.00 \pm 1.62^{c,B}$	$51.00 \pm 0.88^{c,C}$	$39.65 \pm 0.07^{c,A}$
	PPe	$48.91 \pm 0.24^{b,C}$	$36.55 \pm 1.19^{b,A}$	$49.88 \pm 0.54^{c,C}$	$40.46 \pm 0.10^{c,B}$
	EH	$48.99 \pm 1.12^{b,C}$	$36.85 \pm 0.22^{b,A}$	$55.62 \pm 0.33^{d,D}$	$40.42 \pm 0.24^{d,B}$

[a–e]Values with different superscript small letters within the same column at single storage day are significantly different ($P < 0.05$).

[A–D]Values with different capital letter in a row are significantly different for each probiotic microorganism.

[1]Values are mean ± SD (n = 3). Data are adapted from our previous publication with reproducing permission from Elsevier under license no. 5716611111587 (Mudgil et al., 2023b).

[2]UC = unfermented control; LPA = *Lactiplantibacillus argentoratensis* MF000943; LiF = *Limosilactobacillus fermentum* MF000944; LPe = *Lactiplantibacillus pentosus* MF000946; PPe = *Pediococcus pentosaceus* MF000957; and EH = *Enterococcus hirae* MF000958.

yet it is significantly lower than those for *L. fermentum*-fermented goat and bovine milk ($P \leq 0.05$).

The differences in DH% values of fermented milk of different sources by different bacteria can be attributed to variations in protein by-products in the milk of different animals and differences between species (Soleymanzadeh et al., 2016). In addition, the release of proteases by the microorganisms for protein hydrolysis can vary and this influences the degree of hydrolysis of proteins into large peptides, and thus shorter peptides and free AA (Hou et al., 2017). Overall, the hydrolysis of proteins and peptides increased throughout the storage duration and this is in agreement with several studies as residual microbial exo-proteases even during refrigerated storage can cause further protein degradation (Moslehishad et al., 2013a).

Several previous studies that involved measurement of the breakdown of intact proteins to peptides or AA, through fermentation, reported their results in terms of proteolytic activity. Effect of incubation period on the proteolytic activity of camel milk fermented by *Lactobacillus bulgaricus* NCDC, *Lactobacillus fermentum* TDS030603 (incubation time: 0, 3, 6, 9, and 12 h; Solanki et al., 2017), and *Lactobacillus plantarum* KGL3A (incubation time: 0, 6, 12, 24, and 48 h; Dharmisthaben et al., 2021), showed a positive correlation between proteolytic activity in camel milk and incubation time. In addition, similar observations were also reported by (Moslehishad et al., 2013a), in *Lactobacillus rhamnosus* PTCC 1637 fermented bovine and camel milk during 21 d of storage. This effect is associated with the amount of AA needed by the bacteria during their growth through which releasing free NH_3 groups can varies with inoculation levels (Solanki et al., 2017).

Evaluation of In Vitro Antidiabetic Activity Via PAA Inhibition

The antidiabetic activity of different types of fermented milk was evaluated by measuring the PAA inhibitory properties of the samples and their PAA-IC_{50} (μg protein equivalent/mL) values are shown in Table 2. The PAA-IC_{50} of all the unfermented control milk samples was always significantly higher ($P \leq 0.05$) than all the types of fermented milk throughout the storage period. This clearly indicates the capability of PAA inhibition of peptides produced through microbial fermentation in comparison to intact proteins of milk. In fact, a clear trend is noticed with respect to PAA inhibitory activity of unfermented milk throughout the storage. Camel milk showed the highest PAA inhibitory activity throughout the 14-d period, with the maximum activity on d 14 (PAA-IC_{50} 17.30 ± 2.11 μg of protein/mL), that were significantly higher throughout this period. Moreover, unfermented camel milk had lower PAA-IC_{50} values in comparison with several samples of fermented milk of other animals. Again, this evidently reveals the antidiabetic potential of camel milk in comparison to the milk and peptides obtained from other animals, and this has been highlighted in previous studies (Agrawal et al., 2005; Mudgil and Maqsood, 2023).

It was observed that PAA inhibitory activity of fermented milk samples increased during refrigerated storage and could be attributed to release of newer peptides with PAA inhibitory activity across all the types of fermented milks (i.e., bovine, camel, and sheep milk). We found that PPe was the most effective in producing bovine milk peptides with the highest PAA inhibitory activity, the IC_{50} value of its fermented bovine milk was 12.00 ± 2.38 μg of protein/mL on d 0, which decreased further to 9.08 ± 0.68 and 6.01 ± 0.10 μg of protein/mL on d 7 and 14, respectively. Nevertheless, LPA and for LPe fermented bovine milk produced peptides with a promising PAA inhibitory activity that was not significantly different to the activity of PPe fermented bovine milk ($P > 0.05$).

Fermented camel milk showed the most promising PAA inhibitory activity compared with all the other milk provided from different sources and fermented by different microorganisms. Out of all the 60 fermented milk samples of different storage time, LPe–fermented camel milk of d 14 had the lowest PAA-IC_{50} value (2.39 ± 0.51 μg of protein/mL), followed closely by PPe fermented camel milk samples with an PAA-IC_{50} value (3.53 ± 0.39 μg of protein/mL; $P \leq 0.05$), suggesting that LPe and PPe were effective in producing the peptides from camel milk with higher PAA inhibitory potential. In fact, it also derived potent PAA inhibitory peptides from bovine and sheep milk. It was expected that fermented camel milk will show higher antidiabetic activity compared with other types of milk. Several studies have underlined the effectiveness of camel milk-derived peptides in inhibiting diabetes-related enzymes such as PAA, AG, and DPP-IV (Baba et al., 2021a; Ali Redha et al., 2022; Khakhariya et al., 2023; Mudgil and Maqsood, 2023).

Various studies in past few years have explored the antidiabetic functionality of fermented camel and bovine milk through PAA inhibition. In one such study, fermentation of camel and bovine milk was carried out by *Lactococcus lactis* KX881782 (**Lc-KX**), camel milk probiotic strain and *Lactobacillus acidophilus* DSM9126 (**La-DSM**), and the effect of storage time (0, 7, 14, and 21 d) was explored (Ayyash et al., 2018a). Bovine milk fermented by La-DSM and camel milk fermented by Lc-KX showed an PAA inhibition percentage of >40% throughout the storage periods. In addition, prolonged storage positively and significantly influenced the PAA

Table 2. Pancreatic α-amylase (PAA)-IC_{50} (μg protein equivalent/mL) values of various fermented milks during different storage periods at 4°C[1]

Storage period (d)	Organism[2]	PAA-IC_{50} (μg protein equivalent/mL)			
		Bovine	Camel	Goat	Sheep
0	UC	40.31 ± 3.27^{c,B}	23.5 ± 2.71^{d,A}	73.4 ± 6.96^{e,C}	88.8 ± 2.55^{d,D}
	LPA	12.7 ± 2.04^{a,A}	16.0 ± 0.62^{b,A}	15.3 ± 4.03^{a,A}	12.8 ± 1.63^{a,A}
	LiF	21.9 ± 4.14^{b,B}	12.4 ± 0.04^{a,A}	36.1 ± 2.50^{c,C}	22.8 ± 1.70^{c,B}
	LPe	14.7 ± 0.29^{a,A}	19.1 ± 0.19^{c,B}	51.8 ± 1.50^{d,C}	18.3 ± 1.44^{b,B}
	PPe	12.0 ± 0.38^{a,A}	10.9 ± 0.43^{a,A}	25.4 ± 1.80^{b,B}	23.4 ± 0.42^{c,B}
	EH	25.2 ± 2.34^{b,B}	18.3 ± 1.65^{c,A}	32.4 ± 0.68^{c,C}	14.6 ± 0.80^{a,A}
7	UC	33.6 ± 1.60^{e,B}	20.5 ± 0.41^{d,A}	54.7 ± 1.12^{e,C}	57.3 ± 0.60^{e,C}
	LPA	10.7 ± 0.28^{bc,BC}	4.47 ± 0.18^{a,A}	12.9 ± 2.04^{a,C}	8.92 ± 0.10^{c,B}
	LiF	12.0 ± 0.77^{c,C}	3.31 ± 0.24^{a,A}	24.0 ± 0.80^{d,D}	6.63 ± 0.23^{b,B}
	LPe	10.1 ± 0.84^{ab,C}	3.51 ± 0.22^{a,A}	22.3 ± 0.41^{cd,D}	6.71 ± 0.51^{b,B}
	PPe	9.08 ± 0.68^{a,C}	3.48 ± 0.24^{b,A}	15.9 ± 0.67^{b,D}	5.30 ± 0.04^{a,B}
	EH	20.4 ± 0.15^{d,C}	12.2 ± 0.45^{c,A}	21.0 ± 0.30^{c,C}	13.4 ± 0.29^{d,B}
14	UC	29.5 ± 0.06^{d,B}	17.3 ± 2.11^{c,A}	45.8 ± 2.90^{c,C}	52.6 ± 2.34^{c,D}
	LPA	7.76 ± 0.09^{a,B}	3.74 ± 0.18^{b,A}	13.5 ± 2.40^{a,C}	13.4 ± 0.91^{a,C}
	LiF	11.9 ± 0.32^{b,B}	4.08 ± 0.47^{b,A}	14.2 ± 2.53^{a,B}	13.7 ± 0.03^{a,B}
	LPe	7.26 ± 1.15^{a,AB}	2.39 ± 0.51^{a,A}	23.8 ± 3.11^{b,C}	11.9 ± 1.67^{a,B}
	PPe	6.01 ± 0.10^{a,B}	3.53 ± 0.39^{b,A}	15.6 ± 0.34^{a,D}	10.8 ± 0.67^{a,C}
	EH	15.2 ± 1.04^{c,B}	4.28 ± 0.01^{b,A}	15.6 ± 1.51^{a,B}	16.7 ± 1.25^{b,B}

$^{a–e}$Values with different superscript small letters within the same column at single storage day are significantly different ($P < 0.05$).

$^{A–D}$Values with different capital letter in a row are significantly different for each probiotic microorganism. [1]Values are mean ± SD (n = 3).

[2]IC_{50} = the amount of protein equivalent needed to inhibit 50% of enzymes, UC = unfermented control, *Lactiplantibacillus argentoratensis* MF000943 = LPA, *Limosilactobacillus fermentum* MF000944 = LiF, *Lactiplantibacillus pentosus* MF000946 = LPe, *Pediococcus pentosaceus* MF000957 = PPe, and *Enterococcus hirae* MF000958 = EH.

inhibitory activity of fermented camel milk produced by both bacteria, which agrees with our current findings. Yet, this effect was insignificant in fermented bovine samples. Comparing the PAA inhibitory activity of fermented bovine and camel milk, authors reported that camel milk fermented by Lc-KX had significantly greater inhibitory activity ($P < 0.05$). Another study used camel milk probiotic strains *Lactobacillus reuteri* KX881777, *Lactobacillus plantarum* KX881772, and *Lactobacillus plantarum* KX881779 along with a reference strain *Lactobacillus plantarum* DSM2468 to ferment bovine and camel milk for evaluation of their PAA and AG inhibitory activity (Ayyash et al., 2018b). The authors reported that all fermented samples (except camel milk fermented by *Lactobacillus plantarum* KX881772) showed an PAA inhibition percentage of >34% throughout the storage periods. Moreover, prolonged storage also positively and significantly influenced the PAA inhibitory activity (with the exception of fermented bovine milk by *Lactobacillus plantarum* KX881772).

Further, unfermented sheep milk had the highest PAA-IC_{50} values among the other unfermented milk samples, however, upon fermentation, it showed a similar PAA inhibitory potential to fermented bovine milk. The main difference between the PAA inhibitory activity of fermented sheep milk in comparison to the other samples is related to the effect of storage duration. Increasing the storage time, and thus fermentation duration, had a positive effect on the formation of PAA inhibitory peptides from bovine, camel, and goat proteins; that resulted in samples with lower PAA-IC_{50} values by d 14. Nevertheless, sheep milk did not follow this trend. The lowest PAA-IC_{50} values of fermented sheep milk were determined on d 7, those values increased by d 14 suggesting a decrement in PAA activity. This could be due to an increase of proteolysis of peptides into AA after d 14 that resulted in a decrease of PAA inhibitory peptides. Overall, a clear trend was not observed to decide the most efficient bacteria in producing fermented goat milk with PAA inhibitory potential. The EH fermented goat milk of d 7 showed the lowest PAA-IC_{50} value (5.30 ± 0.04 μg of protein/mL) that was significantly lower ($P \leq 0.05$) than the other fermented sheep samples of d 7.

Among the 4 types of milk, fermented goat milk showed the least PAA inhibitory activity. This can be associated with the type and quantities of goat milk proteins. The lowest PAA-IC_{50} value among the fermented goat milk samples was for LPA fermented sample on d 7 (12.90 ± 2.04 μg of protein/mL), yet this was significantly higher than the values of fermented camel and sheep milk of the same bacteria and storage duration. Nevertheless, a study reported the effectiveness of *Lactobacillus plantarum* strains in producing ferment goat milk with considerable PAA inhibitory activity reaching above 40% in some of the samples (Hashemi and Gholamhosseinpour, 2020). The fermen-

tation process in this study involved ultrasonication treatment that significantly affected the PAA inhibitory activity of fermented goat milk and was attributed to further degradation of remaining intact proteins and larger peptides to smaller peptides due to ultrasonic vibrations (Hashemi and Gholamhosseinpour, 2020). Overall, no clear correlation between degree of hydrolysis and PAA inhibitory activity was established. This is attributed to the fact that during the degradation of proteins and peptides with various sequences and lengths are produced, which vary considerably in their biological activity (Mudgil et al., 2023a).

Identification of PAA Inhibitory Peptides: In Silico

Pancreatic α-amylase is made up of 3 distinct parts, known as A, B, and C; in which the most significant active sites are located in part A and between parts A and B. The main active sites of the enzyme are Asp197, Glu233, and Asp300; with a calcium-binding domain comprising of Asp100, Arg158, Asp167, and His201; and a chloride-binding domain involving Arg195, Asn298, and Arg337 (Esfandi et al., 2022). Yet, there are other residues that comprise in the enzyme's vital sites such as Trp58, Trp59, Arg61, Tyr62, His101, Pro163, Asp165, Lys200, Ile235, Asp236, Tyr258, His299, His305, and Ala307 that are considered as hotspot residues that play a definitive role in inhibition (Nadeem et al., 2020).

Table 3 lists all the peptide sequences identified and sequenced from PPe fermented bovine, camel, goat and sheep milk, respectively. All the listed peptides of bovine and camel milk have shown significant ($P < 0.05$) in silico inhibitory activity. A total of 47 bioactive peptides have been identified in the fermented bovine milk, out of those the highest HPEP dock score (−236.534; HPEPDOCK score represents binding energy scores) was for RELEELNVPGE in which it has 8 peptides residues (Arg-1, Glu-2, Leu-3, Glu-4, Asn-7, Val-8, Pro-9, and Glu-11) indicating binding interactions with the enzyme, targeting 8 of the key hotspot residues (Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, and His305*). This is followed by LLYQEPVLGPVRGPFPIIV, with an HPEP dock score of −234.323, in which 12 peptides residues are bound to the enzyme, targeting 6 of the key hotspot residues (Trp58*, Trp59*, Tyr62*, Asp96*, His299*, and His305*). Another peptide YQEPVLGPVRGPFPIIV, with a HPEP dock score of −217.757 was able to show interaction with 10 key hotspots residues and a total of 20 AA on enzymes.

Camel milk is considered a functional dairy product with the most remarkable antidiabetic potential. The fermented sample had 37 bioactive peptides with PAA inhibitory potential. The MMPY peptide showed the highest HPEP dock score (−218.031) with all the residues bound to the enzyme to 9 different key hotspot residues: Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, His299*, Asp300*, and His305*. Two peptides, YDLY and YLDY, were common in fermented bovine and camel milk. Although both peptides have the same composition, the difference in their sequence has remarkably influenced their HPEP dock score. The HPEP dock score of YDLY and YLDY is −162.496 and −212.217, respectively (from fermented camel milk). This suggests the significance of the effect of peptide sequence on the PAA inhibitory potential. Similar observation was marked by (Baba et al., 2021b) for PAA inhibitory peptides derived via pepsin hydrolysis of camel whey protein where peptides LRPFL and LRFPL though has same number of bound residues but had variable peptide ranking score.

A total of 46 bioactive peptides were identified in the fermented goat sample (with a peptide of statistically insignificant activity, $P > 0.05$), among those, RSPK had the highest HPEP dock score (−251.465) with all the residues bound to the enzyme to 4 key hotspot residues (Trp58*, Trp59*, Asp300*, and His305*). The peptide DQHQKAMKPWTQPK also showed a considerable HPEP dock score of −241.724, having Asp-1, His-3, Gln-4, Lys-5, Ala-6, Met-7, Lys-8, Pro-9, Trp-10, Thr-11, Gln-12, and Pro-13 residues involved in binding to 10 key hotspot residues (Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, and His305*).

Fermented sheep milk sample comprised of 45 bioactive peptides (with 3 peptides of statistically insignificant activity, $P > 0.05$), that showed PAA inhibitory activity. The peptides MAQY and MSQF with HPEP dock scores of −219.983 and −218.251, respectively, showed the highest inhibitory potential with all the residues participating in binding to PAA. MAQY and MSQF had the capability of binding to 5 hotspot enzyme residues: Trp58*, Trp59*, Tyr62*, His299*, and Asp300*. In fact, MSQF was capable of binding to His305* as well. Overall, some common peptides between some milk samples. For instance, MMLF was observed between fermented bovine, goat and sheep milk samples. Whereas YDLY, YLDY and YPALVY were common to fermented bovine and camel milk samples, respectively FMLM was found to be common between fermented goat and camel

Milk samples. Similarly, MMLM peptide was common to fermented bovine and sheep milk.

Peptides involving leucine, lysine, cysteine, methionine, glycine, and phenylalanine residues have been reported for their PAA inhibitory activity (Baba et al., 2021b). Peptides with residues proline, leucine, methionine, and cysteine at their N and C terminal ends

Table 3. Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
Fermented bovine milk peptides				
YQEPVLGPVRGPFPIIV	0.003421	Glu-3, Val-5, Leu-6, Gly-7, Pro-8, Val-9, Arg-10, Gly-11, Phe-13, Pro-14, Ile-15, Val-17	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, His305*, Arg337, Lys352, Asp353, Val354, Asp356, Trp357	−217.757
LYQEPVLGPVRGPFP	0.001618	Leu-1, Tyr-2, Gln-3, Pro-5, Val-6, Leu-7, Val-10, Arg-11, Pro-13, Phe-14, Pro-15	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, His305*	−218.467
LLYQEPVLGPVRGPFPIIV	0.001844	Leu-2, Tyr-3, Gln-4, Pro-6, Val-7, Leu-8, Val-11, Arg-12, Gly-13, Phe-15, Pro-16, Ile-18	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300, His305*	−234.323
LYQEPVLGPVRGPFPIIV	0.001844	Leu-1, Tyr-2, Gln-3, Pro-5, Val-6, Leu-7, Val-10, Arg-11, Gly-12, Phe-14, Pro-15, Ile-17	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, His305*	−163.171
RELEELNVPGE	0.005455	Arg-1, Glu-2, Leu-3, Glu-4, Asn-7, Val-8, Pro-9, Glu-11	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, His305*, Arg337, Lys352	−236.534
HIQKEDVPSER	0.002942	His-1, Gln-3, Lys-4, Glu-5, Asp-6, Val-7, Pro-8, Ser-9, Arg-11	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, His305*, Arg337, Lys352, Asp353, Val354, Asp356, Trp357	−171.649
IPNPIGSENSEKTTMPLW	0.001183	Pro-2, Asn-3, Pro-4, Ile-5, Gly-6, Glu-8, Ser-10, Glu-11, Lys-12, Met-15, Pro-16, Leu-17	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, His305*, Arg337	−181.292
PIGSENSEKTTMPLW	0.01677	Pro-1, Ile-2, Gly-3, Glu-5, Glu-8, Lys-9, Thr-11, Met-12, Pro-13, Trp-15	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Thr254, Leu293, Val294, Phe295, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−195.409
IGSENSEKTTMPLW	0.01677	Ile-1, Ser-3, Glu-4, Asn-5, Ser-6, Lys-8, Thr-10, Met-11, Pro-12, Trp-14	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Arg195*, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Lys352, Asp353, Val354, Asp356, Trp357	−210.706
PIGSENSEKTT	0.02281	Pro-1, Ile-2, Gly-3, Glu-5, Ser-7, Glu-8, Lys-9, Thr-11	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, Arg337	−156.388
SEKTTMPLW	0.006044	Ser-1, Glu-2, Lys-3, Met-6, Pro-7, Leu-8	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, Arg337	−112.224
APSFSDIPNPIGSENSEKTTMPLW	0.001855	Ser-3, Phe-4, Ile-7, Pro-8, Asn-9, Pro-10, Ile-11, Ser-13, Glu-14, Asn-15, Ser-16, Lys-18, Thr-20, Met-21, Pro-22, Trp-24	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Lys352, Asp353, Val354, Asp356, Trp357	−209.360
SDIPNPIGSENSEKTTMPLW	0.001614	Pro-4, Asn-5, Pro-6, Ile-7, Gly-8, Glu-10, Ser-12, Glu-13, Lys-14, Met-17, Pro-18, Leu-19	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Arg337	−134.743
CGAGGV	0.02335	Cys-1, Ala-3, Gly-4, Val-6	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*	−155.754
GPPGAI	0.01551	Pro-2, Pro-3, Gly-4, Ile-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−202.146
CVNGSLAC	0.01024	Val-2, Asn-3, Ser-5, Leu-6, Ala-7, Cys-8	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−221.489
CAGPAP	0.002369	Cys-1, Ala-2, Pro-4, Pro-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−128.685
IDNLAAGLVGGAGVVPGE	0.0123	Asn-3, Leu-4, Ala-6, Gly-7, Leu-8, Val-9, Ala-12, Val-14, Val-15, Pro-16, Glu-18	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, His305*, Arg337, Lys352	−147.779
MMLM	0.00962	Met-1, Met-2, Leu-3, Met-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−161.844

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
NRAM	0.002999	Asn-1, Arg-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−195.573
QKDFSH	0.06738	Lys-2, Asp-3, Phe-4, Ser-5, His-6	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp356	−153.853
YPALVY	0.001392	Tyr-1, Pro-2, Ala-3, Leu-4, Val-5	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−180.195
RNAM	0.003212	Arg-1, Asn-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−212.217
MMFL	0.01401	Met-1, Met-2, Phe-3, Leu-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−169.575
NHTW	0.01151	Asn-1, His-2, Thr-3, Trp-4	Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, His299*, Asp300*	−193.696
SWVR	0.01265	Ser-1, Trp-2, Val-3, Arg-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−183.538
RNGLPE	0.00398	Arg-1, Asn-2, Gly-3, Leu-4, Pro-5	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−169.745
NRAM	0.002999	Asn-1, Arg-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−161.844
WRPLN	0.001355	Trp-1, Arg-2, Pro-3, Asn-5	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−161.954
WHLTY	0.02817	His-2, Leu-3, Thr-4, Tyr-5	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Tyr342	−162.446
YDPY	0.002956	Tyr-1, Asp-2, Pro-3, Tyr-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−210.248
MMLF	0.008335	Met-1, Met-2, Leu-3, Phe-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−161.954
YDPY	0.002956	Tyr-1, Asp-2, Pro-3, Tyr-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−218.619
NRAM	0.002999	Asn-1, Arg-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−161.844
MFSQ	0.004489	Met-1, Phe-2, Ser-3, Gln-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−152.795
ENVGGPL	0.01429	Glu-1, Val-3, Gly-5, Pro-6, Leu-7	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337	−127.770
HDHLLF	0.01421	Asp-2, His-3, Leu-4, Leu-5, Phe-6	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337, Tyr342	−142.925
CCVMLNPLW	0.003324	Val-3, Met-4, Leu-5, Pro-7, Leu-8, Trp-9	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*, His305*, Lys352, Asp356	−161.921
YLDY	0.03011	Tyr-1, Leu-2, Asp-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−197.125
RFNH	0.004296	Arg-1, Phe-2, Asn-3, His-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−205.706
HAAGSLLP	0.008596	Ala-2, Ala-3, Gly-4, Leu-6, Leu-7, Pro-8	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, His305*, Arg337	−141.069
PAAY	0.002182	Pro-1, Ala-2, Ala-3, Tyr-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−153.853
DLNPAR	0.0009266	Leu-2, Asn-3, Pro-4, Ala-5, Arg-6	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−171.060
YPPA	0.0001625	Tyr-1, Pro-2, Pro-3, Ala-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−147.502
RNAM	0.003212	Arg-1, Asn-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−211.629
MPAAASR	0.002264	Met-1, Pro-2, Ala-4, Ala-5, Ser-6, Arg-7	Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp356	−172.322
YDLY	0.03011	Tyr-1, Asp-2, Leu-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−175.229
Fermented camel milk peptides				
YLEELHRLN	0.0111	Glu-3, Glu-4, His-6, Arg-7, Leu-8, Asn-9	His15, Phe17, Glu18, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337, Tyr342	−147.983
ENIDELKDTR	0.03379	Asp-4, Glu-5, Leu-6, Lys-7, Asp-8, Arg-10	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu223, Asn298, His299*, Asp300*, Arg337	−135.328

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
NIDELKDTR	0.02491	Asp-3, Glu-4, Leu-5, Lys-6, Asp-7, Arg-9	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu223, Asn298, His299*, Asp300*, Arg337	−192.07
IEEQQQTEDEQQDK	0.04773	Gln-4, Gln-5, Gln-6, Thr-7, Glu-10, Gln-11, Gln-12, Asp-13, Lys-14	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu223, His299*, Asp300*, His305*, Lys352	−158.33
IMEQQQTEDEQQDK	0.03689	Met-2, Gln-4, Gln-5, Gln-6, Glu-10, Gln-11, Gln-12, Asp-13, Lys-14	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352	−213.822
DVPKTKETIIPK	0.00394	Asp-1, Val-2, Pro-3, Lys-4, Thr-5, Glu-7, Thr-8, Ile-10, Pro-11, Lys-12	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu223, His299*, Asp300*, His305*, Arg337, Lys352, Asp356	−170.863
ETIIPK	0.02887	Glu-1, Thr-2, Ile-4, Pro-5, Lys-6	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu223, His299*, Asp300*, His305*, Lys352, Asp356	−154.068
PKLLHPVPQESSF	0.003102	Pro-1, Lys-2, Leu-3, Leu-4, His-5, Val-7, Pro-8, Gln-9, Glu-10, Ser-11	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu223, His299*, Asp300*, His305*, Tyr342, Lys352, Asp353, Val354, Asp356, Trp357	−126.81
QPLGYFK	0.00066	Gln-1, Pro-2, Leu-3, Gly-4, Tyr-5, Phe-6	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, Arg337	−147.394
FMLM	0.01025	Phe-1, Met-2, Leu-3, Met-4	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Tyr342	−155.354
CHNLDELKDTR	0.0125	Cys-1, His-2, Asn-3, Leu-4, Leu-7, Lys-8, Asp-9, Thr-10, Arg-11	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, Arg303, His305*, Tyr342, Asp356	−165.998
YDLF	0.02495	Tyr-1, Asp-2, Leu-3, Phe-4	Trp58*, Trp59*, Asp300*, His305*	−143.199
RLER	0.008988	Arg-1, Leu-2, Glu-3, Arg-4	His15, Phe17, Gln41, Val42, Trp58*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, Arg337	−172.391
RTPLDELKDTR	0.00304	Arg-1, Thr-2, Pro-3, Leu-4, Glu-6, Lys-8, Asp-9, Thr-10, Arg-11	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−158.616
APLY	0.001292	Ala-1, Pro-2, Leu-3, Tyr-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−136.483
YPALVY	0.001392	Tyr-1, Pro-2, Ala-3, Leu-4, Val-5	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−161.921
MMPY	0.0009681	Met-1, Met-2, Pro-3, Tyr-4	Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*, His305*	−218.031
CQGR	0.0008481	Cys-1, Gln-2, Gly-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−175.687
YLDY	0.03011	Tyr-1, Leu-2, Asp-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−212.217
CAVVPNY	0.0006948	Ala-2, Val-3, Val-4, Pro-5, Asn-6, Tyr-7	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, His305*	−185.443
NSAR	0.002401	Asn-1, Ser-2, Ala-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−89.158
RPPPPVAM	0.0001697	Pro-2, Pro-3, Pro-4, Val-6, Ala-7, Met-8	His15, Phe17, Gln14, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, His305*	−154.893
RPPPPLGF	0.0002239	Arg-1, Pro-2, Pro-3, Pro-5, Leu-6, Phe-8	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Arg303, His305*, Tyr342, Asp356	−181.431
PMAVY	0.001621	Pro-1, Met-2, Ala-3, Val-4	Trp58*, Trp59*, Asp300*, His305*, Lys352, Asp353, Val354, Asp356, Trp357	−175.563
QMCNPVPK	0.0005299	Met-2, Cys-3, Asn-4, Pro-5, Val-6, Pro-7	Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*	−134.823
PTHLW	0.002256	Pro-1, Thr-2, His-3, Leu-4	Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, His299*, Asp300*	−175.229
NGGHGV	0.06675	Asn-1, Gly-2, His-4, Val-6	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Tyr342	−200.708

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
EPSGAGFQ	0.02761	Glu-1, Gly-4, Ala-5, Phe-7, Gln8	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337	−181.463
DSAN	0.008793	Asp-1, Ser-2, Ala-3, Asn-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−174.926
FAEAC	0.04897	Ala-2, Glu-3, Ala-4, Cys-5	Trp59*, His305*, Lys352	−196.617
YDLY	0.03011	Tyr-1, Asp-2, Leu-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−162.496
EVLVEM	0.01496	Val-2, Leu-3, Val-4, Glu-5, Met-6	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−177.59
YAVY	0.01025	Tyr-1, Ala-2, Val-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−136.953
FDELLF	0.01642	Phe-1, Asp-2, Glu-3, Leu-4, Phe-6	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−155.749
MLELLM	0.02648	Met-1, Leu-2, Leu-4, Leu-5, Met-6	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Tyr342	−152.89
QLAEGFQ	0.1152	Gln-1, Leu-2, Ala-3, Glu-4	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337	−143.413
YVEY	0.04414	Tyr-1, Val-2, Glu-3, Tyr-4	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−163.588
Fermented goat milk peptides				
LYQEPVLGPVRGPFPILV	0.00178	Leu-1, Tyr-2, Gln-3, Pro-5, Val-6, Leu-7, Pro-13, Phe-14, Pro-15, Ile-16, Leu-17, Val-18	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, His305*	−166.792
VLSLSQPK	0.006529	Leu-2, Leu-4, Ser-5, Gln-6, Pro-7, Lys-8	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Tyr342, Lys352, Asp356	−172.195
VKETMVPK	0.01047	Val-1, Lys-2, Met-5, Val-6, Pro-7, Lys-8	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Arg303, His305*, Asp356	−156.692
MPFPK	0.0005242	Met-1, Pro-2, Phe-3, Pro-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−178.094
VPQRDMPIQA	0.003449	Val-1, Arg-4, Met-6, Pro-7, Ile-8, Gln-9	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, His305*, Arg337	−159.329
IHPFAQAQS	0.01124	Pro-3, Ala-5, Gln-6, Ala-7, Ser-9	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp353, Val354, Asp356, Trp357	−122.592
EPVLGPVR	0.003852	Glu-1, Val-3, Leu-4, Pro-6, Val-7, Arg-8	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−197.266
KIHPFAQAQS	0.0046666	Lys-1, Ile-2, His-3, Pro-4, Phe-5, Ala-6	Phe17, Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, His299*, Asp300*	
DQHQKAMKPWTQPK	0.0004035	Asp-1, His-3, Gln-4, Lys-5, Ala-6, Met-7, Lys-8, Pro-9, Trp-10, Thr-11, Gln-12, Pro-13	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, Arg303, His305*, Asp356	−241.724
KPWTQPK	0.0004035	Lys-1, Pro-2, Trp-3, Thr-4, Gln-5, Pro-6	Phe17, Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−158.652
KPWTQPKTNAIP	0.000577	Pro-2, Trp-3, Thr-4, Gln-5, Pro-6, Lys-7, Asn-8, Ala-10, Ile-11, Pro-12	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−176.867
DMESTEVFTKK	0.06007	Asp-1, Met-2, Glu-3, Ser-4, Glu-6, Val-7, Phe-8, Thr-9, Lys-10	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Thr254, Leu293, Val294, Phe295, Asn298, His299*, Asp300*, Arg337	−146.249
KPWTQPKTNAIPYVRYL	0.00124	Lys-1, Pro-2, Trp-3, Thr-4, Gln-5, Pro-6, Ala-10, Ile-11, Pro-12, Val-14, Arg-15, Tyr-16	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu233*, Asn298, His299*, Asp300*, Arg337	−192.108
TPQH	0.0009516	Thr-1, Pro-2, Gln-3, His-4	Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, Asp300*, His305*, Lys352, Asp356	−161.844
PVVLY	0.004375	Pro-1, Val-2, Val-3, Leu-4	Trp58*, Trp59*, Asp300*	−217.62

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
VPEH	0.01278	Val-1, Pro-2, Glu-3, His-4	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Rg337	−147.175
FLDY	0.02495	Phe-1, Leu-2, Asp-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−172.427
YDLM	0.02872	Tyr-1, Asp-2, Leu-3, Met-4	Trp58*, Trp59*, Tyr62*, Asp300*, His305*	−147.888
NRAM	0.002999	Asn-1, Arg-2, Ala-3, Met-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−158.33
NLLR	0.008729	Asn-1, Leu-2, Leu-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−203.989
PETPLT	0.006365	Pro-1, Thr-3, Pro-4, Leu-5	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−141.037
TDPNLQ	0.003242	Thr-1, Asp-2, Pro-3, Asn-4, Leu-5	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Tyr342, Lys352, Asp356	−162.446
FMLM	0.01025	Phe-1, Met-2, Leu-3, Met-4	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Tyr342	−139.486
CPAALS	0.001914	Cys-1, Pro-2, Ala-3, Leu-5, Ser-6	Phe17, Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp356	−216.418
MMLF	0.008335	Met-1, Met-2, Leu-3, Phe-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−155.277
EWFSSQ	0.009479	Trp-2, Phe-3, Ser-4, Ser-5, Gln-6	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−166.77
NLRL	0.01086	Asn-1, Leu-2, Arg-3, Leu-4	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, Tyr342	−211.038
RAPRW	0.0004926	Arg-1, Ala-2, Pro-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, Arg303, His305*, Asp356	−143.436
YLSH	0.04393	Tyr-1, Leu-2, Ser-3, His-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−147.261
PAKLE	0.001523	Pro-1, Ala-2, Lys-3, Leu-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*	−129.58
FDVVPK	0.003281	Phe-1, Val-3, Val-4, Pro-5, Lys-6	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Arg303, His305*, Asp356	−194.148
YEAVVLL	0.01082	Glu-2, Ala-3, Val-4, Val-5, Leu-6, Leu-7	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337	−157.696
RSPK	0.001121	Arg-1, Ser-2, Pro-3, Lys-4	Trp58*, Trp59*, Asp300*, Arg303, His305*, Lys352, Asp356	−251.465
WSGN	0.01397	Trp-1, Ser-2, Gly-3, Asn-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−167.615
RPGPNLTVY	0.001792	Arg-1, Pro-2, Pro-4, Leu-6, Val-8, Tyr-9	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, His305*, Arg337, Lys352, Asp356	−149.269
MTPY	0.001073	Met-1, Thr-2, Pro-3, Tyr-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−171.158
HVAGGGHM	0.01731	His-1, Val-2, Ala-3, Gly-5, His-7, Met-8	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, His305*, Arg337, Lys352, Asp356	−126.185
DPALHPR	0.0005729	Asp-1, Ala-3, Leu-4, His-5, Pro-6, Arg-7	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Tyr231, Glu233*, His299*, Asp300*, Arg337	−188.897
QVVALR	0.004875	Val-2, Val-3, Ala-4, Leu-5, Arg-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−156.438
EPLTPY	0.0007807	Pro-2, Leu-3, Thr-4, Pro-5, Tyr-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−188.006
PTAVSTY	0.06235	Pro-1, Thr-2, Val-4, Tyr-7	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−168.059
MQAN	0.001436	Met-1, Gln-2, Ala-3, Asn-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−180.048
QLALTY	0.009748	Gln-1, Leu-2, Ala-3, Leu-4, Tyr-6	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−161.746

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with *α-amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
RNSM	0.003332	Arg-1, Asn-2, Ser-3, Met-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352, Asp356	−207.615
Fermented sheep milk peptides				
LYQEPVLGPVRGPFPILV	0.007818	Leu-1, Tyr-2, Gln-3, Pro-5, Val-6, Leu-7, Pro-13, Phe-14, Pro-15, Ile-16, Leu-17, Val-18	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, His305*	−164.365
YQEPVLGPVRGPFPILV	0.001226	Glu-3, Val-5, Leu-6, Gly-7, Pro-8, Val-9, Pro-12, Phe-13, Pro-14, Ile-15, Leu-16, Val-17	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, His305*, Arg337	−157.618
VLSLSQPK	0.006529	Leu-2, Leu-4, Ser-5, Gln-6, Pro-7, Lys-8	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Tyr342, Lys352, Asp356	−156.923
VKETMVPK	0.01047	Val-1, Lys-2, Met-5, Val-6, Pro-7, Lys-8	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, His299*, Asp300*, Arg303, His305*, Asp356	−204.829
YQEPVLGPVRGPFPILT	0.003655	Glu-3, Val-5, Leu-6, Gly-7, Pro-8, Val-9, Arg-10, Pro-12, Phe-13, Pro-14, Ile-15	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−160.124
FESEEQQQTEDELQDK	0.00534	Phe-1, Glu-2, Ser-3, Glu-4, Gln-7, Gln-8, Glu-10, Asp-13, Glu-12, Leu-13, Gln-14, Lys-16	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Tyr231, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−165.17
HQTEDELQDK	0.0281	Glu-4, Asp-5, Glu-6, Leu-7, Gln-8, Lys-10	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Tyr231, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−138.435
IHPFAQAQS	0.01124	Pro-3, Ala-5, Gln-6, Ala-7, Ser-9	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp353, Val354, Asp356, Trp357	−151.296
VKETMVPKHK	0.004658	Glu-3, Met-5, Val-6, Pro-7, Lys-8, His-9	His15, Phe17, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg303, His305*, Arg337, Lys352, Asp356	−214.981
REQEELNVVGETVESLSSSEESITHINK	0.01656	Arg-1, Gln-3, Leu-6, Asn-7, Val-8, Val-9, Glu-11, Val-13, Leu-16, Ser-17, Ser-18, Ser-19, Glu-21, Ser-22, His-25, Ile-26, Asn-27, Lys-28	His15, Phe17, Glu18, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Tyr342, Lys352, Asp356	−142.377
YSGH	0.02395	Tyr-1, Ser-2, Gly-3, His-4	Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, Asp300*, His305*	−144.651
MAQY	0.001969	Met-1, Ala-2, Gln-3, Tyr-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*	−218.251
MSQF	0.01364	Met-1, Ser-2, Gln-3, Phe-4	Trp58*, Trp59*, Tyr62*, His299*.Asp300*	−219.983
KASW	0.01129	Lys-1, Ala-2, Ser-3, Trp-4	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp356	−140.959
CTSSPQ	0.01387	Ser-3, Ser-4, Pro-5, Gln-6	Trp58*, Trp59*, Tyr62*, Asp300*, His305*, Lys352, Asp353, Val354, Asp356, Trp357	−170.277
FMPY	0.001534	Phe-1, Met-2, Pro-3, Tyr-4	Trp58*, Trp59*, Asp300*, His305*	−157.251
YTDN	0.02316	Tyr-1, Thr-2, Asp-3, Asn-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−146.11
FMLF	0.01271	Phe-1, Met-2, Leu-3, Phe-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−158.436
MEASCPK	0.005779	Met-1, Glu-2, Ala-3, Pro-6, Lys-7	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg303, His305*, Arg337, Asp356	−140.822
EQLDSQ	0.01526	Glu-1, Leu-3, Asp-4, Ser-5, Gln-6	His15, Gln41, Val42, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, His299*, Asp300*, Arg337	−120.487
LRLR	0.008275	Leu-1, Arg-2, Leu-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−178.607
RGSSSPE	0.005217	Arg-1, Gly-2, Ser-4, Ser-5, Pro-6, Glu-7	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352	−137.882

Continued

Table 3 (Continued). Potential molecular interaction between identified bioactive peptide from *Pediococcus pentosaceus* MF000957 fermented bovine milk with α-*amylase* enzyme using Pepsite 2 and HPEP dock score[1]

Peptide	Pepsite2 analysis: α-amylase (1SMD)			HPEP dock score
	P-value	Bound peptide residue	Bound amylase residue	
PYLAR	0.002027	Pro-1, Tyr-2, Leu-3, Arg-5	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−175.218
VVYV	0.03603	Val-1, Val-2, Tyr-3, Val-4	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Arg337	−146.491
MMLM	0.00962	Met-1, Met-2, Leu-3, Met-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−135.332
KTLVPQ	0.006314	Leu-3, Val-4, Pro-5, Gln-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−139.731
HANAGAAGH	0.06155	His-1, Asn-3, Gly-5, Ala-6, Ala-7	His15, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asp197*, Glu223, Asn298, His299*, Asp300*, Arg337	−190.015
QVLAPLSGNAVQ	0.001161	Val-2, Leu-3, Ala-4, Pro-5, Leu-6, Gly-8, Asn-9, Ala-10, Val-11, Gln-12	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−160.579
MMLF	0.008335	Met-1, Met-2, Leu-3, Phe-4	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−178.001
EVGGVPK	0.00219	Glu-1, Val-2, Gly-4, Val-5, Pro-6, Lys-7	His15, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Asp96*, Val98, Arg195*, Asp197*, His299*, Asp300*	−171.457
QRGGGSGF	0.1425	Gln-1, Arg-2, Gly-3, Ser-6	Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Asp96*, Arg195*, Asn298, His299*, Asp300*, Arg337	−153.683
LRFGAR	0.00295	Leu-1, Arg-2, Phe-3, Ala-5, Arg-6	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, Tyr342	−126.583
YDLM	0.02872	Tyr-1, Asp-2, Leu-3, Met-4	Trp58*, Trp59*, Tyr62*, Asp300*, His305*	−150.304
MNLN	0.01528	Met-1, Asn-2, Leu-3, Asn-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−188.613
YYLSY	0.04737	Tyr-2, Leu-3, Ser-4, Tyr-5	Trp58*, Trp59*, Asp300*, His305*, Lys352, Asp356	−146.11
YTDN	0.02316	Tyr-1, Thr-2, Asp-3, Asn-4	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−172.978
FSKAAY	0.0189	Ser-2, Lys-3, Ala-4, Ala-5, Tyr-6	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, Arg303, His305*, Lys352, Asp353, Val354, Asp356, Trp357	−192.532
ESGGSVGTQ	0.2525	Glu-1, Val-6, Thr-8, Gln-9	His15, Phe17, Gln41, Trp58*, Trp59*, Tyr62*, Phe295, Asp297, Asn298, His299*, Asp300*, Arg337	−133.927
YTVAFE	0.02443	Tyr-1, Thr-2, Val-3, Phe-5, Glu-6	His15, Phe17, Gln41, Val42, Ser43, Pro44, Trp58*, Trp59*, Tyr62*, Gln63, Asp96*, His101*, Leu165, Arg195*, His299*, Asp300*, Arg337	−179.787
PRAGSY	0.00448	Pro-1, Arg-2, Ala-3, Gly-4, Ser-5	Trp58*, Trp59*, Tyr62*, Arg195*, Asp197*, Glu233*, His299*, Asp300*, His305*, Lys352, Asp356	−145.573
EGTGVH	0.174	Glu-1, Gly-2, Gly-4, His-6	Phe17, Glu18, Trp58*, Trp59*, Tyr62*, Asp300*, His305*, Tyr342, Lys352	−180.67
CYDD	0.02519	Cys-1, Tyr-2, Asp-3, Asp-4	His15, Gln41, Val42, Tyr62*, Asp96*, Arg195*, Asp197*, Tyr231, Glu233*, Asn298, His299*, Asp300*, Arg337	−128.291
EAAAAVR	0.03158	Glu-1, Ala-2, Ala-4, Val-6, Arg-7	Phe17, Trp58*, Trp59*, Tyr62*, His299*, Asp300*, His305*, Lys352	−155.069
NLVNGH	0.01467	Leu-2, Val-3, Asn-4, Gly-5, His-6	Trp58*, Trp59*, Tyr62*, Asp197*, Glu233*, Asp300*	−150.194
VMGR	0.004973	Val-1, Met-2, Gly-3, Arg-4	Trp58*, Trp59*, Tyr62*, His299*, Asp300*	−136.704

*Potential hotspots for α-amylase inhibition if bounded by the peptide.
[1]The HPEPDOCK score represents binding energy scores and is available at the HPEPDOCK server (http://huanglab.phys.hust.edu.cn/hpepdock/). Pepsite2 is available at http://pepsite2.russelllab.org/.

Table 4. Summary of binding interactions between α-amylase (PDB ID: 1SMD) and selected peptides from fermented milk of different farm animals

			Protein-peptide binding interactions						
			Hydrophobic interaction		Hydrogen bond			Salt bridge	
Source	Peptide sequence	Binding affinity (kcal/mol)	Peptide residue involved	α-Amylase residue involved	Peptide residue involved	α-Amylase residue involved	Bond length (Å)	Peptide residue involved	α-Amylase residue involved
Acarbose		−9.1	—	Trp58*, Trp59*, Tyr62*, Tyr151, Leu162, Ser163, Leu165, Ile235, Gly306	—	Gln63	3.16	—	—
						Lys200	2.78		
						Asp300*	2.97		
						His305*	2.61		
Bovine	YQEPVLGPVRGPFPIIV	−10.5	Tyr1, Gln2, Glu3, Pro4, Val5, Pro8, Val9, Arg10, Pro12, Phe13	Asn53, Pro54, Trp58*, Trp59*, Gln63, Gly104, Ala106, Val107, Ser163, Leu165, Asp300*, His305*, Glu349, Lys352, Val354, Asp356, Trp357	Pro8	Gln63	2.95	Arg10	Asp300*
					Arg10	Asp300*	2.58	Arg10	Asp300*
					Arg10	Asp300*	2.75		
	RELEELNVPGE	−10.3	Glu2, Leu3, Glu4, Glu5, Leu6, Asn7, Val8, Pro9, Gly10, Glu11	Trp59*, Tyr151, Leu162, Ser163, Leu165, Lys200, His201, Glu233*, Ile235, Asp300*, His305*, Gly306, Lys352, Asp356, Trp357	Glu2	Lys352	2.63	Glu2	Lys352
					Glu4	Lys352	2.64	Glu4	His305*
					Gly10	Tyr151	3.04	Glu4	Lys352
Camel	IMEQQQTEDEQQDK	−11.4	Ile1, Met2, Glu3, Gln5, Gln6, Thr7, Asp9, Glu10, Gln11, Asp13, Lys14	Asp147, Ile148, Tyr151, Leu162, Ser163, Trp269, Asp300*, Aeg303, Gly304, His305*, Gly306, Ala307, Gly308, Gly309, Ala310, Ser311, Phe348, Gly351, Lys352, Asp353	Gln5	Tyr151	2.95	—	—
					Met2	Ser163	2.86		
					Gln6	Asp300*	3.13		
					Asp9	Gly308	2.82		
					Asp9	Gly309	2.69		
					Asp13	Ala310	2.60		
					Asp13	Ser311	2.80		
					Lys14	Gly351	2.69		
					Glu10	Asp353	2.97		
	MMPY	−8.2	Met1, Met2, Pro3, Tyr4	Trp59*, Gln63, Ser163, Arg195*, Asp197*, Glu233*, Asp300*, His305*, Gly306, Lys352, Val354, Asp356, Trp357	Met1	Lys352	3.07	—	—
					Tyr4	Arg195*	2.78		
					Tyr4	Glu233*	2.68		
					Tyr4	Gly306	3.02		
Goat	DQHQKAMKPWTQPK	−11.3	Asp1, Gln4, Lys5, Met7, Lys8, Pro9, Trp10, Thr11, Gln12, Pro13, Lys14,	Asn53, Pro54, Trp58*, Trp59*, Tyr62*, Gln63, Gly104, Ala106, Leu162, Ser163, Gly164, Leu165, Asp300*, Arg303, His305*, Gly306, Glu349, Val354, Asp356, Trp357	Asp1	Trp357	2.97	Lys5	Glu349
					Gln4	Asn53	2.73	Lys5	Glu349
					Lys8	Val354	2.75		
					Lys8	Asp356	2.72		
					Pro9	Ser163	2.99		
					Thr11	Gln63	2.89		
					Pro13	Gly306	3.11		
					Lys14	His305*	2.86		
	CPAALS	−8.7	Cys1, Pro2, Ala3, Ala4, Leu5, Ser6	Trp58*, Trp59*, Tyr62*, Leu162, Leu165, Asp197*, His201, Glu233*, His299*, Asp300*, His305*, Gly306, Asp356	Cys1	His201	3.03	—	—
					Cys1	Gly233	3.12		
					Cys1	Glu233*	2.75		

Continued

Table 4 (Continued). Summary of binding interactions between α-amylase (PDB ID: 1SMD) and selected peptides from fermented milk of different farm animals

			Protein-peptide binding interactions							
			Hydrophobic interaction		Hydrogen bond			Salt bridge		
Source	Peptide sequence	Binding affinity (kcal/mol)	Peptide residue involved	α-Amylase residue involved	Peptide residue involved	α-Amylase residue involved	Bond length (Å)	Peptide residue involved	α-Amylase residue involved
Sheep	VKETMVPKHK	−9.6	Lys2, Glu3, Thr4, Met5, Val6, Pro7, Lys8, His9, Lys10	Trp59*, Tyr151, Ser163, Lys200, His201, Glu233*, Ile235, Asp300*, Gly304, His305*, Lys352, Val354, Asp356, Trp357	Lys2 Glu3 Lys8 Lys8 His9	Val354 Lys352 Gly304 His305* Tyr151	2.68 2.60 2.75 2.84 2.89	Lys2 Glu3 His9	Asp356 His305* Glu233*
	HANAGAAGH	−10.3	His1, Ala2, Asn3, Ala4, Gly5, Ala6, Ala7, His9	Trp58*, Trp59*, His101, Tyr151, Leu162, Leu165, Lys200, His201, Glu233*, Ile235, Glu240, Asp300*, His305*, Gly306, Lys352, Val354, Asp356, Trp357	His9 His9	Trp59* Lys352	2.85 2.84	His9	Asp356

*Potential hotspots for α-amylase inhibition if bounded by the peptide.

were common in peptides with PAA inhibitory activity. Among the key peptides discussed earlier, 3 peptides (MMPY, MAQY and MSQF) have a methionine residue and one peptide (LLYQEPVLGPVRGPFPIIV) has leucine at their N terminus. In addition, leucine, lysine, methionine, glycine, and phenylalanine residues were present in those key peptides. Over 20 peptides with tyrosine at their N terminus were identified. Residues with aromatic AA can form aromatic-aromatic interactions with PAA by hydrogen bonds, van der Waals and electrostatic interactions (Ali Redha et al., 2022).

SAR Analysis Using Molecular Docking

Inhibitors of carbohydrate digesting enzymes (i.e., PAA and AG) are the primary target for diabetes management. Although not completely known, it is believed that inhibitors of these enzymes interact within the enzyme's active sites and inhibit the enzyme-substrate complex formation causing disruption of enzymatic functions. Therefore, research aimed toward identifying compounds making strong interactions with catalytic binding sites of these enzymes is gaining particular interest from drug developers. In silico analysis via molecular docking has emerged as an efficient approach for prediction of binding affinity of various compounds with drug targets and therefore is being used extensively drug development. Therefore, to get deeper insight into the enzyme binding affinity of fermented milk-derived peptides molecular docking studies were conducted on selected peptides with strongest binding affinities toward different binding sites of PAA as predicted by Pepsite2 and HPEPDock scores.

Following SAR analysis of peptides, a total of 8 peptides (i.e., YQEPVLGPVRGPFPIIV and RELEELNVPGE from fermented bovine milk, IMEQQQTEDEQQDK and MMPY from fermented camel milk, DQHQKAMKPWTQPK and CPAALS from fermented goat milk and VKETMVPKHK and HANAGAAGH from fermented sheep milk) were selected for molecular docking. The interaction between peptides and enzyme are presented in Table 4 and Figure 1a–e. Ability of various peptides to make hydrogen bonds with PAA indicate their high potential for inhibition via creation of a sliding barrier causing hindrance with substrate binding sites (Siow and Gan, 2016).

As shown in Table 4 all the selected peptides could make hydrogen bonds with the target enzyme. Peptide YQEPVLGPVRGPFPIIV from fermented bovine milk formed hydrophobic interactions, hydrogen bonding and salt bridges with the catalytic binding residue using Asp300 in addition hydrophobic interaction were seen with catalytic triad of PAA using Trp58, Trp59, and His305 with a binding energy of −10.5 kcal/mol,

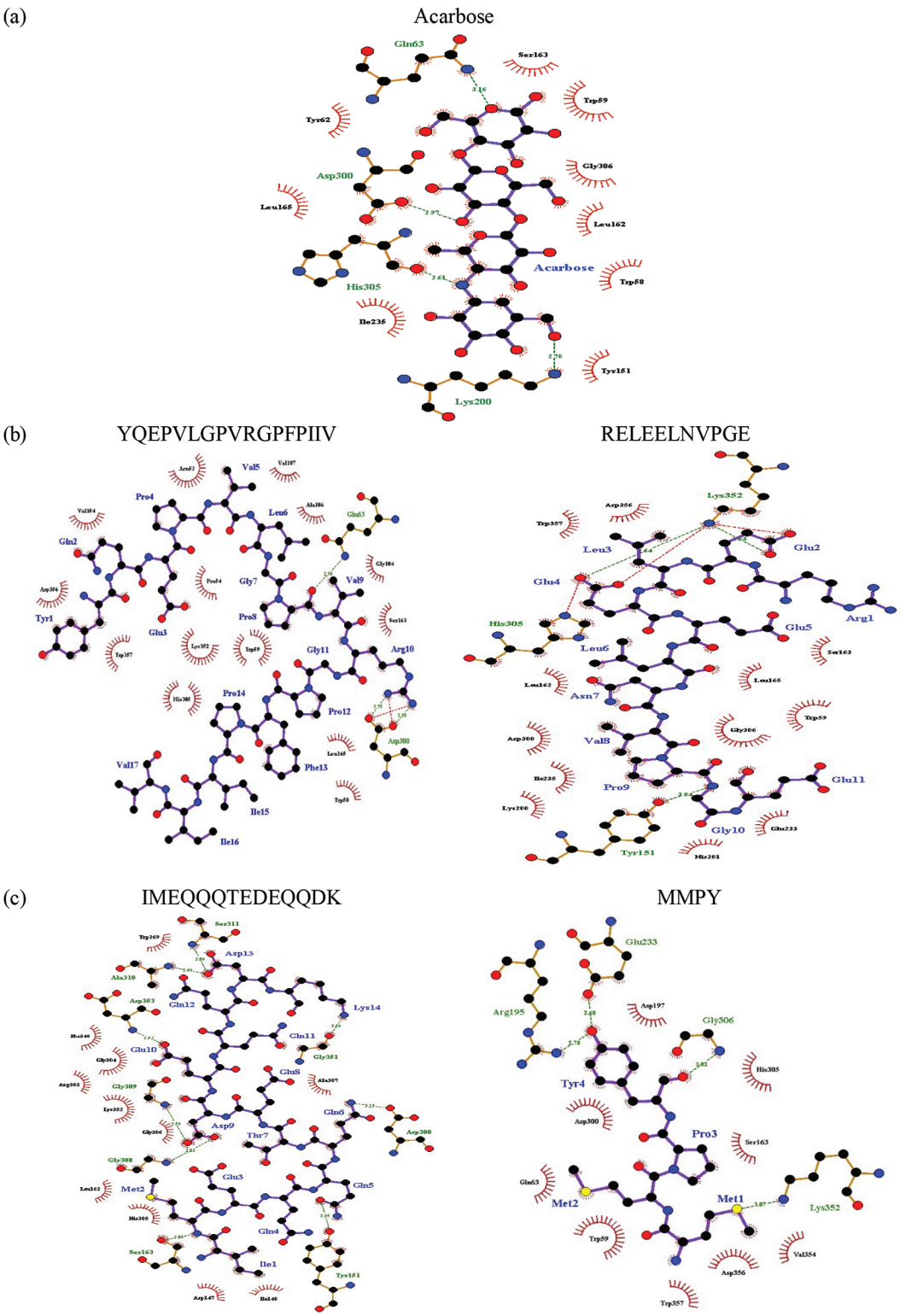

Figure 1. Binding interactions between α-amylase and (a) acarbose as well as peptides from (b) bovine, (c) camel, (d) goat, and (e) sheep. Red circle indicates oxygen atom; blue circle, nitrogen atom; black circle, carbon atom; yellow circle, sulfur atom; purple line, peptide; brown line, α-amylase; red dotted line, salt bridge; green dotted line with number, hydrogen bond and bond length (Å) between the proton donor and acceptor; brick red eyelashes, hydrophobic interaction.

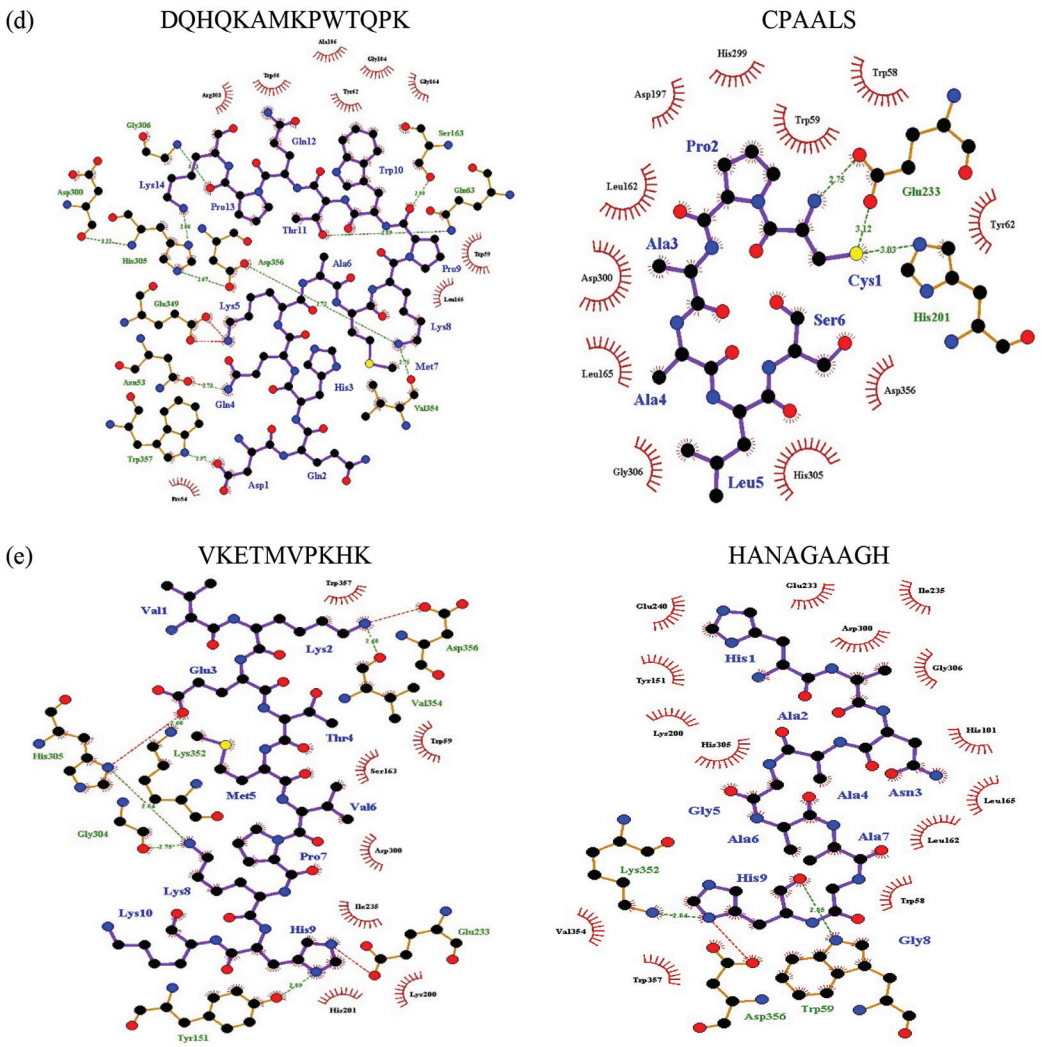

Figure 1 (Continued). Binding interactions between α-amylase and (a) acarbose as well as peptides from (b) bovine, (c) camel, (d) goat, and (e) sheep. Red circle indicates oxygen atom; blue circle, nitrogen atom; black circle, carbon atom; yellow circle, sulfur atom; purple line, peptide; brown line, α-amylase; red dotted line, salt bridge; green dotted line with number, hydrogen bond and bond length (Å) between the proton donor and acceptor; brick red eyelashes, hydrophobic interaction.

which is comparatively higher than commercial drug acarbose. Similarly, peptide RELEELNVPGE with a binding energy of −10.5 kcal/mol showed hydrophobic interaction with PAA using Trp59, Glu233, Asp300 and His305. This peptide also showed salt bridge formation using His305 of catalytic triad but no hydrogen bonding with any hotspot. Overall, maximum number of hydrogen bonds (9) were shown by fermented camel milk-derived peptide IMEQQQTEDEQQDK with maximum binding energy of −11.4 kcal/mol among all peptides analyzed. This peptide interacted hydrophobically with catalytic pockets using Asp300, and His305. Further, peptide MMPY showed hydrophobic interactions with PAA hotspots using Trp59, Arg195, Asp197, Glu233, Asp300, and His305. Interestingly both selected peptides from fermented camel milk showed no interaction with PAA using salt bridges. Furthermore, second to IMEQQQTEDEQQDK fermented goat milk-derived peptide DQHQKAMKPWTQPK indicated maximum binding energy of −11.3 kcal/mol and hydrogen bonding with 8 different residues on enzyme. This peptide also showed second most hydrophobic interactions with catalytic hotspots on PAA via interactions with Trp58, Trp59, Tyr62, Asp300, and His305. Another peptide CPAALS from fermented goat milk showed no interaction using salt bridge but interacted with enzyme using maximum hydrophobic number interactions in the catalytic hotspots involving Trp58, Trp59, Tyr62, Asp197, Glu233, His299, Asp300, His305. Hydrogen bonding using His201, Gly233, and Glu233 was also

observed for the following peptide with a binding energy of −8.7 kcal/mol. Both the peptides (i.e., VKETMVPKHK and HANAGAAGH from fermented sheep milk) showed a binding energy of −9.6 and −10.3 kcal/mol, respectively. Both the peptides showed all 3 types of interactions (i.e., hydrophobic, hydrogen bonds, and salt bridges with PAA; Table 4). These results are comparable to those obtained by (Fadimu et al., 2022), where various peptides obtained from alcalase and flavorzyme hydrolyzed lupin hydrolysates indicated binding energy with −6.9 to −9.1 kcal/mol. Peptides obtained in our study has indicated significantly higher binding energy and more interaction in comparison to theirs.

CONCLUSIONS

The increased demand for food-derived bioactive peptides to manage lifestyle-related disorders is attributed to their superior health benefits. Peptides from milk proteins, specifically bovine and camel milk, are studied widely for their bioactive properties. This study, however, compared peptides obtained from probiotic fermentation of 4 milk types (cow, camel, goat, and sheep). Results showed significantly higher PAA inhibition in fermented camel milk after refrigerated storage, with LPA and PPe producing the most potent peptides. Molecular docking studies revealed a strong affinity of peptides from camel and goat milk proteins toward the PAA catalytic site. These findings suggest that LPA and PPe from camel milk could be effective starter cultures for functional foods with antidiabetic properties. Furthermore, probiotic fermentation-derived bioactive peptides hold potential for large-scale production of nutraceuticals for diabetic patients. However, further studies on specific peptides are needed for validation using in vitro, cell lines, and in vivo approaches.

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