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Identification of novel α-glucosidase and ACE inhibitory peptides from Douchi using peptidomics approach and molecular docking

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ABSTRACT

In this study, the effect of Douchi extract (*DWE*) on α-glucosidase and angiotensin-converting enzymes (ACE) were investigated, and several novel peptides with inhibitory activity against α-glucosidase and ACE were identified using peptidomics approach based on UPLC-MS/MS. The average inhibition rates of *DWE* on α-glucosidase and ACE were 73.75–78.10% and 4.56–27.07%, respectively. In the *DWE*, a total of 710 peptides were detected. Two novel peptides with potential inhibitory activity against α-glucosidase were identified using the correlation analysis, database alignment and molecular docking methods. They were DVFRAIPSEVL and DRPSINGLAGAN, with the IC_{50} values of 0.121 and 0.128 mg/mL, respectively. Also, four novel peptides with potential inhibitory activity against ACE were identified: PSSPFTDLWD, EEQDERQFPF, PVPVPVQQAFPF and PSSPFTDL, with IC_{50} values of 1.388, 0.041, 0.761 and 0.097 mg/mL, respectively. These results indicated that combining peptidomics and molecular docking is an effective alternative strategy for rapidly screening numbers of novel bioactive peptides from foods.

1. Introduction

Douchi is one of Chinese most famous traditional condiment (Guo, Zhang, Long, Fu, & Ren, 2023). It is processed from soybeans through soaking, cooking, koji making and fermentation (Chen et al., 2021). Besides high levels of protein, fat, and amino acids, Douchi has many bioactive components, such as isoflavone, peptides, vitamins, etc (Wang et al., 2021). Also, Douchi showed many bio-functionalities, such as antioxidant activity, hypoglycemic activity (Shehzad, Shahzad, Bilal, & Lee, 2020) and hypotensive activity (Baskaran et al., 2017), etc. However, the responsible molecules and the mechanism underlying their beneficial effects remain unclear. The bioactive peptides are suggested as one of the main responsible components that are not thoroughly studied. The screening and identifying of bioactive peptides in Douchi are obtaining more and more attention (Iwaniak, Darewicz, Mogut, & Minkiewicz, 2019). Thus, our present study aims to rapidly screen hypoglycemic and hypotensive peptides from Douchis, in order to reveal the related compounds and the functionalities mechanism.

Foods contain a large number of peptides, but the content of active peptides is very low. The purification and separation processes of active peptides involve multiple laborious technologies, such as centrifugation, membrane separation, ethanol precipitation and multidimensional column chromatography, etc (Zhang, Tatsumi, Ding, & Li, 2006). The separated peptides with potential biological activity will be subsequently identified by mass spectrometry and artificially synthesized to verify the activity. For example, Zhong et al. (2021) et al. applied affinity-ultrafiltration membranes to obtain four peptide ultrafiltration fractions derived from Skipjack tuna hydrolysate and then identified a novel xanthine oxidase inhibitory peptide ACECD by HPLC-MALDI-TOF/TOF-MS. Yu et al. (2022) identified three novel hypoglycemic peptides (HPFR, VY and SFLLR) from Douchi through step-wise purification technology including C18 Sep-Pak technique, size exclusion chromatography, and semi-preparative liquid chromatography, and then the peptides were sequenced by UPLC-MS/MS. Obviously, the stepwise separation has low efficiency (Amin, Kusnadi, Hsu, Doerksen, & Huang, 2022).

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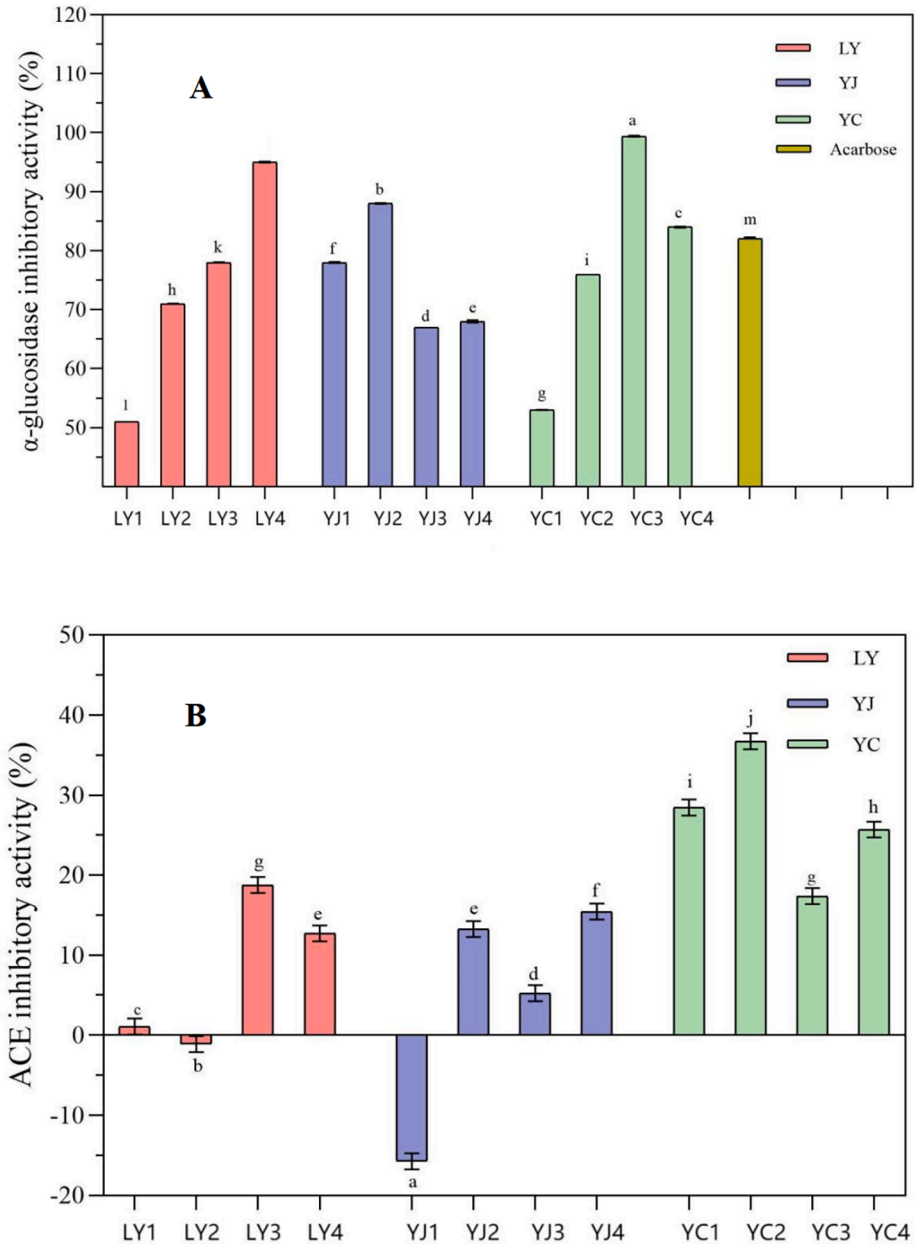


Fig. 1. Inhibitory activities of *DWE* on α-glucosidase (A) and ACE (B) (The concentration of acarbose is 0.01 %, w/w). LY indicates the Liuyang Douchi, YJ indicates the Yangjiang Douchi, YC indicates the Yongchuan Douchi. LY1-4 indicate 4 different samples of Liuyang Douchi, YJ1-4 indicate 4 different samples of Yangjiang Douchi, YC1-4 indicate 4 different samples of Yongchuan Douchi.

To improve the efficiency, the latest strategy is combining peptidomics and molecular docking. Peptidomics can determine large numbers of peptide's sequences at one sampling which reduce the purification process. The molecular docking can exclude most sequences with low possibility of activity, which may reduce the workload of peptide artificial synthesis for verification. Furthermore, the molecular docking can also be used to analyze structure–activity relationship to explain the peptides' bioactive mechanism (Fabre, Combier, & Plaza, 2021). However, preparative chromatography is still required. For example, Martini, Solieri, Cattivelli, Pizzamiglio, and Tagliazucchi (2021) applied the UHPLC to obtain water-soluble low-molecular-weight peptide from Parmigiano-Reggiano cheese and determined 415 unique peptides using peptidomic approach based on HR-MS..Combined with the molecule docking, six peptides with potential DPP-IV and α-glucosidase inhibitory activity were screened, followed by artificial synthesis and activity determination *in vitro*. The tripeptide IPP was identified as potential antihypertensive peptide and the peptide APFPE was identified as a novel DPP-IV inhibitor (IC_{50} = 49.5 ± 0.5 μmol/L). Shi, Wei, and Huang (2021) extracted low-molecular-weight peptides from cheese by ultrafiltration membrane and determined a total of 534 peptides by peptidomic approach based on Nano-HPLC-MS/MS. Then, eleven potential bioactive peptides were screened out according to the results of molecular docking, and four novel ACE inhibitory peptides (YPFPGPIH, LKNWGEGW, RELEEIR and HPHPHLS) were identified after artificially synthesis and verification *in vitro*. Zhang et al. (2022) used gel filtration chromatography (Sephadex G-15) to obtain peptides' fraction from Pea protein hydrolysates, and determined 543 peptides by peptidomics approach. Eight peptides with high molecular docking scores were artificial synthesized and the activity was determined *in vitro*. Finally, four strong inhibitory peptides (IPYWTY, IPYWT, LPNYN, and LAFPGSS) against DPP-IV were identified.

Based on the aforementioned methodologies for rapidly screening

Table 1
The results of correlation coefficient, database alignment and molecular docking score of potential α-glucosidase inhibitory peptides screened.

No.	Sequence	r	Known activity in the database	Zdock score	Toxicity prediction results
1	IQAEGGLT[a]	–	Alpha-glucosidase inhibitor	813.795	Non-Toxin
2	ALPEEVIQHTF	0.581*	/	1012.563	Non-Toxin
3	DFYNPKAGRIS	0.587*	/	1271.955	Non-Toxin
4	DRPSIGNLAGAN	0.576*	/	864.809	Non-Toxin
5	DVFRAIPSEVL	0.609*	/	1005.688	Non-Toxin
6	IVTVEGGLSVISPKW	0.627*	Alpha-glucosidase inhibitor	1018.981	Non-Toxin
7	LVIDGRGHL	0.645*	/	834.456	Non-Toxin
8	TFSEYPPLGR	0.588*	Alpha-glucosidase inhibitor	1213.188	Non-Toxin
9	TLNSLTLPALRQF	0.608*	/	1227.057	Non-Toxin

*Representing significant correlation.
/Not recorded in the database.
[a] Peptide sequence come from reference (Vilcacundo, Martínez-Villaluenga, & Hernández- Ledesma, 2017).

functional peptides, we have designed our study as follows. A number of Douchi samples were collected, and the corresponding water extract of Douchi (*DWE*) were prepared. Then, the inhibitory activity of the *DWE* against α-glucosidase and ACE of *DWE* were determined, and peptidomics technology based on UPLC-MS/MS was applied to determine sequence and corresponding content of the peptides. Then, the correlation analysis between peptides' content and the inhibitory activity data was performed, the peptides positive significant correlated with the inhibiting rate were selected as potential bioactive peptides, followed by database alignment and molecular docking that excluded peptides with low docking scores for α-glucosidase or ACE. Finally, the ones showed high docking scores were synthesized artificially, and their activities were analyzed *in vitro*. The IC_{50} was determined to verify the inhibitory activity of the identified peptides.

This new strategy greatly reduced the workload of traditional multi-step purification process. In the meantime, the correlation analysis increased the possibility of discovering novel bioactive peptides. Thus, this study was a meaningful exploration in methodology of rapid screening and identifying novel active peptides in foods.

2. Materials and methods

2.1. Materials

Liuyang Douchi (Guo et al., 2023), Yangjiang Douchi and Yongchuan Douchi (Chen, Li, Shi, Mao, & Du, 2013) were used in this study, as they are the most typical ones in China. Twelve samples were collected, with four subsamples for each. The Douchi were collected from local supermarkets. The samples were labeled as LY1–LY4, YJ1–YJ4, and YC1–YC4, respectively. The Douchi samples were stored at −20 °C before use.

2.2. Methods

2.2.1. Extracting of peptides from Douchi

One gram of Douchi were grounded into powder in liquid nitrogen, and 30 mL of water was added to the powder. The mixture was vortexed, extracted with ultrasonication for 10 min, freezing centrifugation at 4 °C 20000 × g for 30 min (TG25KR, Dongwang Instruments Ltd., Changsha, China), and the supernatant was collected (noted as *DWE*) and stored at 4 °C.

The *DWE* were analyzed for α-glucosidase and ACE inhibitory activity within 24 h. The *DWE* were also desalted and underwent peptidomics analysis based on Ultra High-Pressure Nanoscale Liquid Chromatography with a Orbitrap Fusion mass spectrometer (UPLC-MS/MS).

2.2.2. Determination of α-glucosidase inhibitory activity of DWE

The α-glucosidase inhibitory activity was determined according to the method described by Dong et al (2015). The reaction mixture was composed of 30 uL of 5 mM p-nirophenyl α-D-glucopyranoside (PNPG, Sigma-Aldrich Ltd., Shanghai, China), 50 uL of 0.1 M phosphate buffer (pH 6.8), 40 μL of a sample, and 30 uL α-glucosidase (*Saccharomyces cerevisiae*) solution (0.2 U/mL) (Yuanye biology Ltd., Shanghai, China). This reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 100 μL of 0.2 M sodium carbonate solution. The absorbance readings (A) were recorded at 405 nm by a micro-plate reader (DNM-9602, Beijing Perlong Medical New Technology Co., Ltd., Beijing, China) and compared to a control which had 40 uL of buffer in place of the sample. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$Inhibition of\ \alpha-glucosidase\ activity(\%) = \frac{(A_{control}-A_{sample})}{A_{control}} \times 100\%$$

The 0.01 % Acarbose (Sigma-Aldrich Ltd., Shanghai, China) was used as a positive control (Wu, Zhou, Chen, Li, Wang, & Zhang, 2017).

2.2.3. Measurement of ACE-inhibitory activity of DWE

The ACE inhibitory activity was measured by the modified Cushman method (Chin et al., 2019). It was calculated as follows:

$$Inhibitory\ of\ ACE\ activity(\%) = 1 - \frac{A-C}{B-D} \times 100\%$$

where A is the fluorescence intensity of the ACE (from rabbit lung, Sigma-Aldrich Ltd., Shanghai, China) solution with ACE inhibitors; B is the fluorescence intensity of ACE solution without ACE inhibitors; C is the fluorescence intensity of the ACE inhibitors solution; and D is the fluorescence intensity of the buffer. The fluorescence intensity were recorded at 228 nm by a micro-plate reader (DNM-9602, Beijing Perlong Medical New Technology Co., Ltd., Beijing, China).

2.2.4. The peptidomics analysis based on UPLC-MS/MS

The *DWE* was desalted through the Sep-Pak C_{18} column (Waters, USA). The desalting column was equilibrated with 400–600 μL of 1 % acetonitrile (containing 0.1 % TFA, v/v). After sampling, another 200 μL of 0.5% acetonitrile (containing 0.1 % TFA, v/v) was added to clean the column and to wash off the residual salts. Then 300 μL of 80% acetonitrile (containing 0.1 % TFA, v/v) was used to collect the peptides (Zhang, Zhan, Chen, & Sun, 2018).

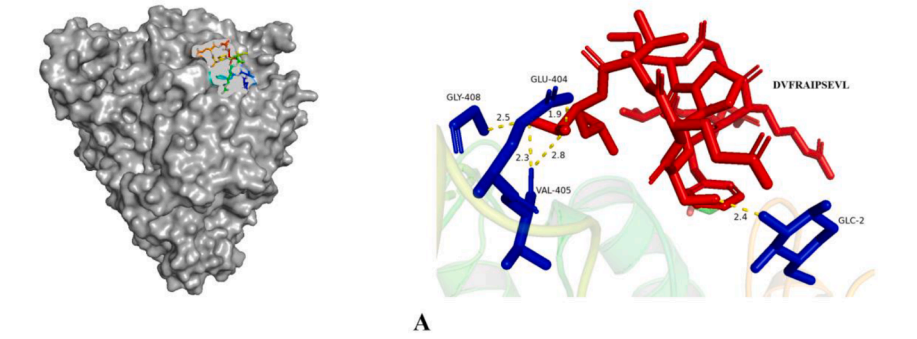
All chromatographic analyses were conducted on an UltiMate 1000RSLCnano system (Thermo Scientific, USA), equipped with a C-18 trap column (75 μm × 250 mm, 3 μm). The mobile phase A was 0.1 % formic acid (v/v). The mobile phase B was acetonitrile containing 0.1 % formic acid (v/v). Peptides were separated over 112 min with a gradient from 0 to 90 % solvent B at a flow rate of 500 nL/min (Lee et al., 2016).

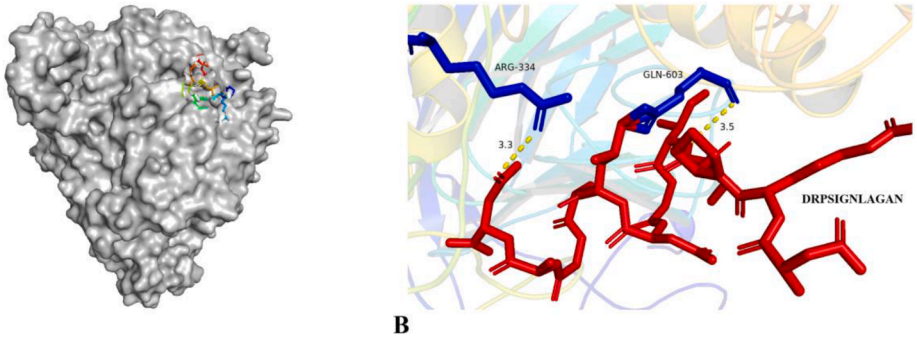
A full MS scan (400–1,600 *m/z*) was acquired in the mass spectrometer with the resolution of 120, 000. The minimum ion intensity value for MS/MS was 50,000, the maximum ion introduction time for MS/MS was 100 ms, the AGC control was 1.0×10^5, and the parent ion selection window was 1.6 Dalton (Dong, Mei, Zhu, Liu, & Huang, 2012). The mass spectrometry was searched with MaxQuant software (version 1.6.5.0), and quantitative analysis was performed using the iBAQ algorithm.

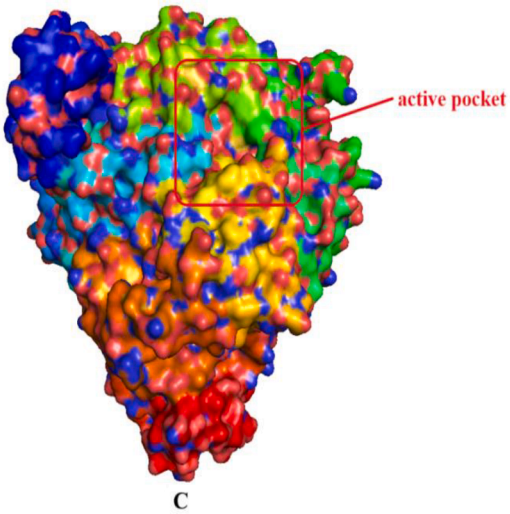
2.2.5. Screening of novel active peptides from Douchi

Briefly, the content of peptides in the *DWE* detected by UPLC-MS/MS analysis was correlated with the inhibition rates of α-glucosidase and ACE. Peptides with significant positive correlations ($p < 0.05$) were screened as potential active peptides and were further underwent toxicity prediction and sequence alignment.

The peptide toxicity was predicted by the ToxinPred software (website: https://webs.iiitd.edu.in/raghava/toxinpred/design.php),







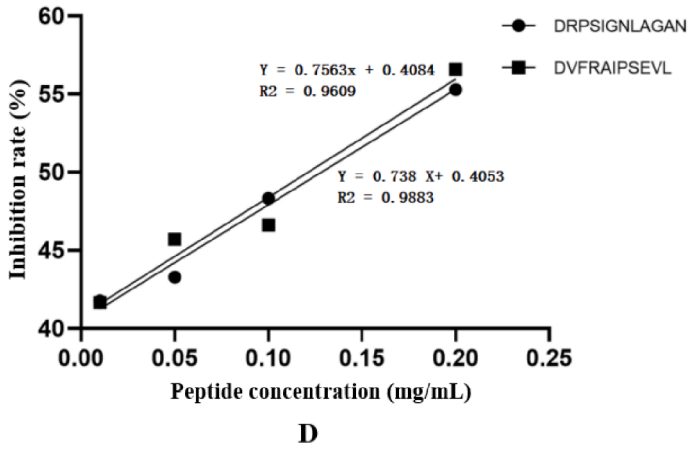


Fig. 2. Molecular docking position analysis of α-glucosidase inhibitory peptides and inhibitory activity of synthetic peptides on enzyme. A and B showed the docking structure of ligands DVFRAIPSEVL and DRPSIGNLAGAN in complex with α-glucosidase (PDB code: 2QMJ), respectively (yellow dashed lines indicate hydrogen bonds; blue structures represent amino acids residues of α-glucosidase; red structures represent peptide ligand). C, Predict active pocket of α-glucosidase (PDB code: 2QMJ). D, Inhibition rate (%) of synthesis peptides on α-glucosidase.

Table 2
The results of correlation coefficient, database alignment and molecular docking score of potential ACE inhibitory peptides screened.

No.	Sequence	r	Known activity in the database	Zdock score	Toxicity prediction results
1	QIGLF[a]	–	ACE inhibitor	1095.907	Non-Toxin
2	PSEVEAVFKPLGPEVGELRV	0.673*	ACE inhibitor	1132.777	Non-Toxin
3	PVPVQQAFPF	0.672*	/	1534.462	Non-Toxin
4	DRDSYNLHPGDAQRIPAGTTY	0.668*	ACE inhibitor	1094.332	Non-Toxin
5	NNPFKFLVPPQESQKR	0.644*	ACE inhibitor	1122.391	Non-Toxin
6	PLDLTSFVLH	0.642*	ACE inhibitor	1341.364	Non-Toxin
7	VISQIPSQVQELAFPG	0.613*	ACE inhibitor	1069.855	Non-Toxin
8	PIYSNKLGKFF	0.612*	ACE inhibitor	1625.917	Non-Toxin
9	WGPLVNPESQQGSPR	0.602*	ACE inhibitor	1513.316	Non-Toxin
10	IGTLAGANSLLNALP	0.597*	ACE inhibitor	869.563	Non-Toxin
11	PSSPFTDLWDPRRVGD	0.594*	ACE inhibitor	1378.387	Non-Toxin
12	**YEAGVVPPGARFEPPR**	0.592*	ACE inhibitor	1268.014	Non-Toxin
13	RPSIGNLAGANSLLNALPE	0.589*	ACE inhibitor	841.897	Non-Toxin
14	RPSIGNLAGANSLLNALP	0.588*	ACE inhibitor	835.843	Non-Toxin
15	DRPSIGNLAGANSLLNALP	0.587*	ACE inhibitor	888.602	Non-Toxin
16	PSSPFTDL	0.582*	/	1100.951	Non-Toxin
17	TPMIGTLAGANSLLNALP	0.582*	ACE inhibitor	867.791	Non-Toxin
18	**EGEQPRPFPFP**	0.581*	ACE inhibitor	1325.17	Non-Toxin
19	PFSFLVPPQESQR	0.581*	ACE inhibitor	1588.148	Non-Toxin
20	PSSPFTDLWD	0.765*	/	1551.674	Non-Toxin
21	EEQDERQFPF	0.747*	/	1200.589	Non-Toxin

/Not recorded in the database.
*Representing significant correlation.
[a] Peptide sequence come from reference (Yu et al., 2011).

and the sequence alignment was performed using the online database BIOPEP-UWM (available from: https:// biochemia.uwm.edu.pl/) and AHTPDB (available from: https://crdd.osdd.net /raghava/ahtpdb/). The peptides with no toxicity and had not been included in these databases were further investigated with molecular docking. The ones with docking scores higher than that of reference peptide sequence were selected for further synthesis and bioactivity analysis *in vitro* described in 2.2.2 and 2.2.3. The α-glucosidase inhibitory peptide IQAEGGLT (IC_{50} value: 0.86 mg/mL) (Vilcacundo, Martínez-Villaluenga, & Hernández-Ledesma, 2017) and ACE inhibitory peptide QIGLF (IC_{50} value: 0.42 mg/mL) (Yu, Zhao, Liu, Lu, & Chen, 2011) were used as the reference peptide sequence. Their corresponding docking scores were 813.80 and 1095.901 in present study, respectively.

2.2.6. Molecular docking analysis

2.2.6.1. Preparation and treatment of the receptor structure. The natural crystal structure of ACE (PDB Code: 1O8A, Homo sapiens) and α-glucosidase (PDB Code: 2QMJ, Homo sapiens) was used as the initial model for molecular docking analysis (Tian, Li, & Li, 2021).

2.2.6.2. Preparation and treatment of the ligand structure. The PEP-FOLD3 (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) online server was used for peptide structure prediction (Zhao et al., 2019), the three-dimensional structures of the peptides were constructed and stored in PDB format.

2.2.6.3. Molecular docking. The POCASA prediction platform (https://altair.sci.hokudai.ac.jp/g6/service/pocasa/) was used to predict ligand binding domains (active pockets) (Seifert et al., 2016).

Protein docking software ZDOCK (https://zdock.umassmed.edu/) was employed, and ligand molecules were docked with receptor active sites. The predicted interactions with the highest docking score were selected for further analysis of binding hydrogen bonds and amino acid residues using the PyMOL molecular graphics system (PyMOL 2.5, Schrödinger Inc., USA) (Singh, Reddy, & Upadhyay, 2021).

2.2.7. Determination of the activity of the potential bioactive peptides in vitro

Two peptides with potential α-glucosidase inhibitory activity and four peptides with potential ACE inhibitory activities were synthesized by the method of FMOC solid-phase peptide synthesis (Moreira, Wolfe, & Taylor, 2021) in China Peptides Co. Ltd., Shanghai; their inhibitory activity was determined as described above, and the IC_{50} was calculated (Fabre, Combier, & Plaza, 2021).

2.3. Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean ± SD. Analysis of variance was performed using SPSS 22 software (International Business Machines Corporation, USA) to examine the differences among treatments at $P < 0.05$.

3. Results and discussion

3.1. Inhibitory rates of DWE against α-glucosidase and ACE

The inhibitory rates of *DWE* of Douchi against α-glucosidase are shown in Fig. 1A. The inhibition rate of *DWE* against α-glucosidase was higher than 50%. Interestingly, the LY4, YJ2 and YC3 had significantly higher inhibitory activity against α-glucosidase than the positive control − 0.01% of acarbose that is equivalent to the amount that a person usually consumes ($p < 0.05$) (Laar et al., 2005).

The inhibitory rates of Douchi extracts against ACE are shown in Fig. 1B. The inhibitory activity of YC2 (36.74%) was significantly higher than others ($p < 0.05$). The overall inhibitory activity of group YC was higher than that of groups LY and YJ. This result is consistent with Su et al (2016). who reported that the ACE inhibition rate (27.9%) of Yongchuan douchi.

3.2. Non-targeted peptidomics analysis of Douchi

Endogenous peptides present in Douchi extracts were detected by peptidomics analysis based on UPLC-MS/MS. In total, 2024 peaks were detected from mass spectrometry. After data processing, 710 peptides were identified (Figure S1). Considering the precursor proteins, identified peptides were divided into 60 categories, which were mainly peptides from glycinin G2 and β-conglycinin (Figure S1). Glycinin and β-conglycinin are two major soybean allergenic globulins that account for 70–80% of the total seed globulin fraction. When soybean was

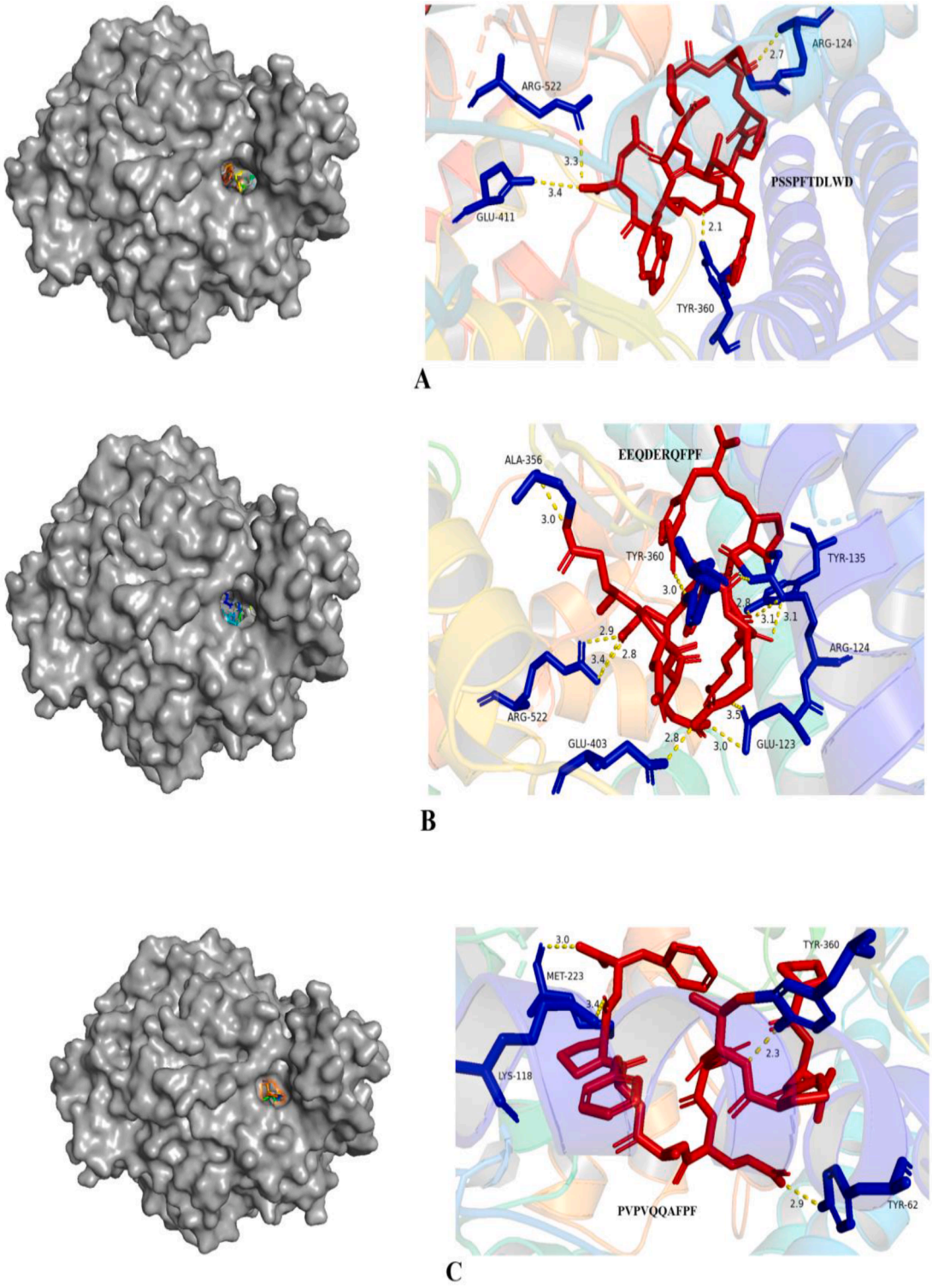
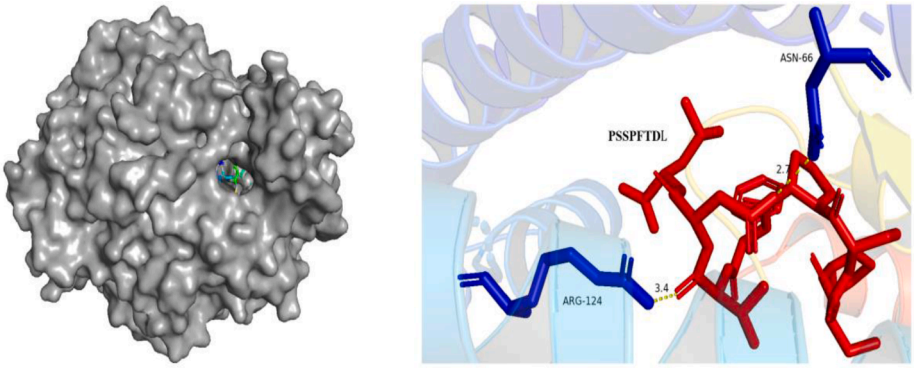


Fig. 3. Molecular docking position analysis of ACE inhibitory peptides and inhibitory activity of synthetic peptides on enzyme. A ~ D showed the docking structure of ligands PSSPFTDLWD, EEQDERQFPF, PVPVQQAFPF, and PSSPFTDL in complex with ACE (PDB code:1O8A), respectively (yellow dashed lines indicate hydrogen bonds; blue structures represent amino acids residues of α-glucosidase; red structures represent peptide ligand). E, Active pocket of ACE (PDB code:1O8A). F, Inhibition rate (%) of synthesis peptides on ACE.

fermented, it was shown that the β-conglycinin a-subunit 1, β-conglycinin a-subunit, glycinin G1, and 2S albumin was specifically degraded and the hydrolysis sites were located on the surface of the molecule or at the mobile disordered region (Shirotani et al., 2021).

3.3. Identification of potential α-glucosidase inhibitory active peptide in Douchi

Type II diabetes is a chronic metabolic disease difficult to cure and is prone to complications (Wu, et al., 2017). Screening of active



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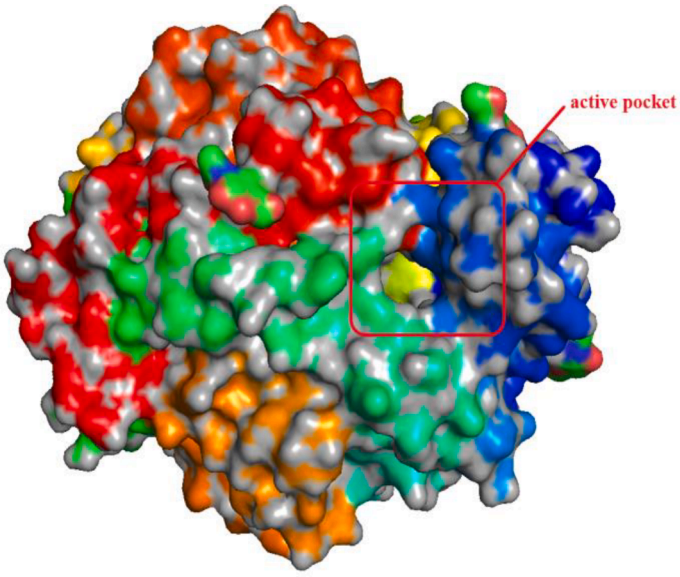

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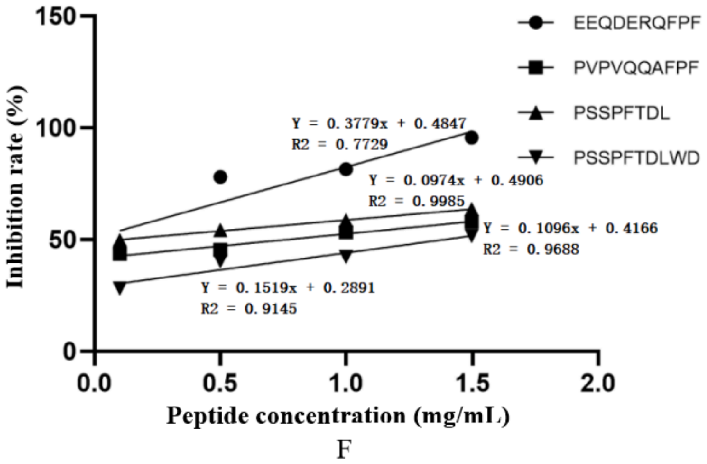

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Fig. 3. (*continued*).

ingredients from foods that could be used in the prevention and treatment of diabetes have become a research hot topic. In this context, several studies have demonstrated the hypoglycemic activity of Douchi (Su & Shang, 2016).

Content of eight peptides was significantly ($p < 0.05$) correlated with inhibition rate of α-glucosidase (Table 1); among them, six peptide sequences had not been included in the database of BIOPEP-UWM and AHTPDB, thus, they might be novel potential bioactive peptides with activity inhibiting α-glucosidase. All identified peptides were non-toxic.

With reference to the binding sites of α-glucosidase inhibitory peptides IQAEGGLT (Zdock score: 813.795) reported in the literature (Vilcacundo et al., 2017), the visualization results of molecular docking showed that only two peptides (DRPSIGNLAGAN, DVFRAIPSEVL) have binding sites located in the active pocket of α-glucosidase (Fig. 2C), indicating these two peptides might be effective inhibitors against α-glucosidase. As shown in Fig. 2B, the DVFRAIPSEVL formed 5

hydrogen bonds with α-glucosidase at residues Glu-404 (bond lengths: 1.9 Å), Val-405 (bond lengths: 2.3, 2.8 Å), Gly-408 (bond lengths: 2.5 Å), and Glc-2 (bond lengths: 2.4 Å). and the DRPSIGNLAGAN formed 2 hydrogen bonds with α-glucosidase at the residues Arg-334 (bond lengths: 3.3 Å) and Gln-603 (bond lengths: 3.5 Å). These residues were also reported locating in the active pocket according to the literature (Sevcík et al., 2010).

These two peptides were subsequently synthesized for their bioactivity validation *in vitro*. As shown in Fig. 2D, the peptides DVFRAIPSEVL and DRPSIGNLAGAN showed promising inhibitory activity against α-glucosidase in a dose-dependent manner. The IC_{50} values of peptides DVFRAIPSEVL and DRPSIGNLAGAN were 0.121 mg/mL and 0.128 mg/mL, respectively. Although they were higher than the reported α-glucosidase inhibitory peptides NSPR (IC_{50} = 0.093 mg/mL) (Zhang, Wang, Wang, Wang, & Li, 2016), they were lower than that of Acarbose (IC_{50} value: 0.69 mg/mL) (Wu, et al., 2017) and of IQAEGGLT (IC_{50} value: 0.86 mg/mL) (Vilcacundo et al., 2017) reported for α-glucosidase inhibiting peptide. Hence, these peptides could be promising α-glucosidase inhibitors.

3.4. Identification of potential ACE inhibitory active peptide in Douchi

The ACE promotes the conversion of type I angiotensin to type II, thereby inhibiting angiotensin activity while simultaneously leading to increase in blood pressure and risk of cardiovascular disease (Ramírez-Sánchez, 2021). Although some ACE inhibitors such as fosinopril (Bhende, Varanasi, & Abbulu, 2020) have rapid and strong efficacy, damage to renal function often occurs when these drugs are used. Peptides isolated from natural foods have demonstrated ACE inhibitory activity and fewer side effects, therefore constituting a research hot area (Zhang, He, Rui, & Simpson, 2021).

Twenty peptides had a significant ($p < 0.05$) positive correlation with ACE inhibition rates (Table 2), which were all non-toxic. In addition, Z-DOCK molecular docking results revealed that 13 out of the 20 peptides had higher docking scores than that reported for the ACE inhibitory peptide QIGLF (1095.907). After further comparison with the database, it was found that only four peptide sequences (PSSPFTDLWD, EEQDERQFPF, PVPVQQAFPF, PSSPFTD) had not been included in the database (BIOPEP-UWM and AHTPDB) and the others had been proven to have ACE inhibitory activity.

The result of visual analysis of molecular docking data of predicted interactions between these four potential ACE inhibitory peptides (PSSPFTDLWD, EEQDERQFPF, PVPVQQAFPF and PSSPFTDL) and ACE are shown in Fig. 3. The PSSPFTDLWD formed 4 hydrogen bonds with ACE at amino acid residues Arg-124 (bond lengths: 2.7 Å), Tyr-360 (bond lengths: 2.1 Å), Arg-522 (bond lengths: 3.3 Å) and Glu-411 (bond lengths: 3.4 Å) (Fig. 3A). The EEQDERQFPF formed 12 hydrogen bonds with ACE at amino acid residues Arg-124 (bond lengths: 3.1, 3.1 Å), Glu-123 (bond lengths: 3.0, 3.5 Å), Glu-403 (bond lengths: 2.8 Å), Tyr-360 (bond lengths: 3.0 Å), Arg-522 (bond lengths: 2.8, 2.9, 3.4 Å), Ala-356 (bond lengths: 3.0 Å) and Tyr-135 (bond lengths: 2.8, 3.2 Å) (Fig. 3B). The PVPVQQAFPF formed 4 hydrogen bonds with ACE at amino acid residues Tyr-360 (bond lengths: 2.3 Å), Tyr-62 (bond lengths: 2.9 Å), Met-223 (bond lengths: 3.0 Å) and Lys-118 (bond lengths: 3.4 Å) (Fig. 3C). The PSSPFTDL formed 2 hydrogen bonds with ACE at amino acid residues Arg-124 (bond lengths: 3.4 Å) and Asn-66 (bond lengths: 2.7 Å) (Fig. 3D). According to predict ligand binding domains (active pockets) (Fig. 3E) and literature (Pan, Cao, Guo, & Bo, 2012), these four peptides (PSSPFTDLWD, EEQDERQFPF, PVPVPVQQAFPF, PSSPFTDL) were located in this active pocket of ACE. These four peptides were thus synthesized for subsequent *in vitro* validation of their bioactivity.

In vitro ACE inhibitory activity of these four synthetic peptides is shown in Fig. 3F. The IC_{50} values of peptides PSSPFTDLWD, EEQDERQFPF, PVPVQQAFPF and PSSPFTDL were 1.388, 0.041, 0.761 and 0.097 mg/mL, respectively. Although they were higher than the reported ACE inhibitory peptides GPAGPAGL (IC_{50} = 0.008 mg/mL) (Kheeree et al., 2020), they were higher than the ACE inhibitory activity of the peptide NMAINPSKENLCSTFCK (IC_{50} value: 2.83 mg/mL) reported in literature (Tu et al., 2018). Among them, EEQDERQFPF and PSSPFTDL were also significantly higher than the ACE inhibitory activity of the peptide IPPGVPYWT (IC_{50} value: 0.4 mg/mL) reported in literature (Sitanggang, Putri, Palupi, Hatzakis, & Budijanto, 2021). These peptides could thus be used as potential ACE inhibitors.

4. Conclusions

Using UPLC-MS/MS-based peptidomics approach, two novel potential α-glucosidase inhibitory peptides and four novel potential ACE inhibitory peptides were identified in Douchi. The screened bioactive peptides were artificially synthesized and their activity were verified *in vitro*. Peptides DVFRAIPSEVL and DRPSIGNLAGAN exhibited good α-glucosidase inhibitory activity with IC_{50} values of 0.121 mg/mL and 0.128 mg/mL, respectively; peptides PSSPFTDLWD, EEQDERQFPF, PVPVQQAFPF and PSSPFTDL had ACE inhibitory activity with IC_{50} value of 1.388, 0.041, 0.761 and 0.097 mg/mL, respectively. These results indicated that combining peptidomics and molecular docking is an effective strategy for rapidly screening bioactive peptides from foods.

CRediT authorship contribution statement

Weidan Guo: Conceptualization, Formal analysis, Writing – original draft. **Yu Xiao:** Conceptualization, Writing – original draft. **Xiangjin Fu:** Supervision, Project administration, Writing – review & editing. **Zhao Long:** Formal analysis, Visualization. **Yue Wu:** Resources, Validation. **Qinlu Lin:** Resources, Validation. **Kangzi Ren:** Writing – review & editing. **Liwen Jiang:** Supervision, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100779.

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