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Research article

Identification of dipeptidyl peptidase IV inhibitory peptides derived from gac seed protein hydrolysate using hydrophilic interaction liquid chromatography and reversed-phase high-performance liquid chromatography

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Abstract

Importance of the work: Dipeptidyl peptidase IV (DPPIV) plays a critical role in Type 2 diabetes. Gac seed proteins (GSPs) can hydrolysate used enzymes.

Objectives: To investigate the potential of gac seed proteins as a functional food for diabetes treatment based on bioactive peptides.

Materials & Methods: The DPPIV inhibitory activities were evaluated using a combination of gastrointestinal proteases. DPPIV inhibitory peptides from GSP hydrolysate were isolated using two sequential bioassay-guided fractions, namely hydrophilic interaction liquid chromatography (HILIC) and reversed-phase high-performance liquid chromatography (RP-HPLC). Peptides in the fraction with the highest DPPIV inhibitory activity were identified using liquid chromatography-mass spectrometry coupled with the de novo sequencing.

Results: The DPPIV inhibitory assay showed that GSPs derived from HILIC-70% acetonitrile had the highest activity. Subsequently, this fraction was separated using RP-HPLC, with fraction 6, fraction 8 and fraction 13 each having high DPPIV inhibitory activity, with 10 identified peptides. Their DPPIV inhibitory activities were further predicted using the BIOPEP database in which DSGL had the highest activity. The molecular docking simulation suggested that the DPPIV inhibitory activities of DSGL could be due to its interaction with key residues in the S2 (Glu205, Arg125) pocket and the catalytic residues in the S1 (Ser630, His740) pocket of DPPIV.

Main finding: GSP hydrolysate showed potential for drug application or as a functional food additive.

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Introduction

Gac seeds have been disregarded in many countries where this fruit is grown (Abdulqader et al., 2019). However, gac seeds have great potential to serve as raw material for medicine preparation, nutraceuticals and functional foods because these seeds contain abundant proteins and bioactive compounds (Ngamsuk et al., 2020). Plant proteins have been known to aid in the impediment of many diseases such as diabetes (Arnoldi et al., 2015). Food-derived bioactive peptides, composed of sequences of 2–50 amino acids, are usually inactive when rooted within the parent protein's structure, but can be released through enzymatic, chemical and microbial hydrolysis (Iavarone et al., 2018; Manzanares et al., 2019).

Diabetes mellitus is a long-term health problem in which hyperglycemia is persistent, leading to other metabolic dysfunctions (Antony and Vijayan, 2021). Of Type 1 and Type 2 diabetes, more than 85% of the global case are caused by the latter (Patil et al., 2015; International Diabetes Federation, 2019). After a meal, the incretins, including glucagon-like-peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) are released from the gut to induce insulin secretion from beta cells and suppress the glucagon secretion from alpha cells. Dipeptidyl peptidase IV (DPPIV) can degrade GLP-1 and GIP, leading to deficient insulin secretion that causes hyperglycemia, commonly found in Type 2 diabetes (Seino and Yabe, 2013). The inhibition of DPPIV activity can preserve the balance in the insulin level that reduces blood glucose (Lyn et al., 2017; Yaribeygi et al., 2019). Natural bioactive peptides from defatted oil seeds or by-products of the food industry have attracted attention because of their inhibitory effect on DPPIV and they can be used as alternatives to chemical drugs for the treatment of diabetes mellitus (Udenigwe and Aluko, 2012; Han et al., 2019). Han et al. (2021) studied oil seed protein hydrolysates (flax seed, rapeseed, sunflower, sesame and soybean) that were hydrolyzed by enzymes and showed potent antidiabetic actions compared to dairy proteins. However, there is a lack of evidence regarding the potential of gac seed-derived bioactive peptides as DPPIV inhibitors.

Active protein hydrolysate is generally composed of peptides with diverse weights, molecular sizes, hydrophobicity and physicochemical properties, with fractionation and purification of the peptides achieved using multidimensional column chromatography, such as solid-phase extraction (SPE), strong cation exchange (SCX), size-exclusion chromatography (SEC), hydrophilic interaction liquid chromatography (HILIC), reversed-phase high-performance liquid chromatography (RP-HPLC) and ion-exchange chromatography (IEC; Liu et al., 2017; Liu et al., 2019; Ngamsuk et al., 2020; Sutopo et al., 2020). Sungthong and Gerdvutgoa (1999) reported that the extract from animal feed purified using SPE was purer in solution than from using other methods. Many researchers have indicated that a combination of fractionation methods aids in achieving better peptide purification (Huang et al., 2012; Ji et al., 2017; Song et al., 2017; Sato et al., 2018). For example, Pujiastuti et al. (2017) reported the RP-HPLC coupled with SCX provided efficient screening of the active ACE peptide. Similarly, Sutopo et al. (2020) reported that the peptide from black cumin seed hydrolysate purified using RP-HPLC and SCX had high levels of ACE inhibitory activity.

The current study investigated the potential of gac seed proteins as a functional food for diabetes treatment based on the bioactive peptides obtained using the two sequential bioassay-guided fractions: HILIC and RP-HPLC. Fraction characterization was carried out by: 1) evaluating their DPPIV inhibitory activity; 2) identifying their peptides using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and automatic de novo sequencing using PEAKS; 3) in silico prediction of the DPPIV inhibitory activities of the peptides using the BIOPEP database; and 4) performing an in silico analysis to stimulate interactions between the peptides identified from the gac seed protein (GSP) hydrolysate and the active pockets of DPPIV.

Materials and Methods

Materials

Gac seeds were collected from the waste from the processed juice in Pingtung, Taiwan. The maturity of the gac fruit was divided based on maturity of ripening and on harvesting in the rainy season. All chemicals and materials used in this research were of analytical grade and obtained from reputable suppliers. Pure water was produced using a PURELAB® water purification system (ELGA LabWater; High Wycombe, UK).

Preparation of gac seed protein powder

Unprocessed seeds were thoroughly washed before drying (hot-air tray dryer; Model HTD-7; Jaw Chuange Machinery

Co., Ltd.; Taoyuan, Taiwan) at 40° C for 36 h. The dried gac seeds were homogeneously pulverized (grinder model RT; Rong Tsong Precision Technology Co.; Taichung, Taiwan) into powder and defatted three times using hexane (gac seed-to-hexane ratio of 1:15, weight per volume) for 30 min at ambient temperature. The residue was exposed to 24 h air-drying in an extraction hood and stored in a desiccator at room temperature until required for use. Protein defatted powders (each 60 mg) were extracted triply using 1% sodium dodecyl sulfate (6 mL) at 0°C in a sonicator (Ultrasonic s-450D; Branson Digital Sonifier®; Rockingham, NH, USA) at 30% amplitude for 3 min. The solutions were centrifuged (15,000×g) (him CT15RE; Hitachi Koki Co., Ltd.; Tokyo, Japan) at ambient temperature for 10 min. The supernatants were lyophilized in a freeze dryer at -80 °C until dry (Panchum Scientific Corp.; Kaohsiung, Taiwan) and then exposed to trichloroacetic acid (10%) in acetone at -20 °C for 24 h to precipitate the protein, which was subsequently centrifuged (15,000×*g*) for 10 min and washed three times with acetone (6 mL). The protein pellet was washed with deionized water, centrifuged (15,000×*g*) for 10 min, lyophilized at -80 °C until dry and kept at -20 °C.

Enzymatic hydrolysis of gac seed proteins

The crude protein from the gac seeds was triply hydrolyzed with pepsin, trypsin and chymotrypsin. A suspension of 10 mg of crude protein pellet in 35 mM sodium chloride (NaCl) was adjusted to pH 2 with 4M hydrochloric acid (HCl). The first hydrolysis was carried out with pepsin (0.2 mg, 250 units/mg) at 37 °C for 24 h. Pepsin inactivation was carried out by adjusting the pH to 8 using 5M sodium hydroxide (NaOH). Subsequently, trypsin (0.2 mg, 250 units/mg) and chymotrypsin (0.2 mg, 40 units/mg) were allowed to further hydrolyze the protein mixture under the same conditions. The peptide solution was centrifuged (10,000×*g*) (him CT15RE; Hitachi Koki Co., Ltd.; Tokyo, Japan) at 10 °C for 10 min to isolate the peptides using an ultrafiltration membrane (MW < 3 kDa; Amicon® Ultra-0.5; Merck Millipore; Darmstadt, Germany) and then the filtrate was lyophilized and kept at -20 °C.

Desalting of gac seed protein hydrolysate using solid-phase extraction

After lyophilization, the high concentration of salt that remained in hydrolysate may interfere with the DPPIV inhibitory assay and LC-MS/MS analysis. To avoid such interference, a desalting step prior to the biological assay and LC-MS/MS analysis is required. HyperSepTM C18 solid-phase extraction (SPE) columns (Thermo Scientific; Rockwood, TN, USA) were used in the desalting of the GSP hydrolysate. The SPE columns were activated by washing the resin with methanol (1 mL) and 95% acetonitrile (1 mL) containing 0.1% methanoic acid, before equilibrating with 1 mL of 5% acetonitrile added with 0.1% methanoic acid. Then, 1 mL of peptide mixture was loaded on the column and washed three times (1 mL of 5% acetonitrile containing 0.1% methanoic acid). Peptide elution was carried out in two stages: first with 1 mL of 50% acetonitrile + 0.1% methanoic acid and then with 80% acetonitrile + 0.1% methanoic acid. The fractions were collected, lyophilized and stored at -20 °C.

Gac seed protein hydrolysate fraction purification using hydrophilic interaction liquid chromatography

Purification of eluted peptides was carried out using HILIC (ZIC®-HILIC SPE; SeQuant; Umea, Sweden). The HILIC columns were activated (1 mL of methanol and 1 mL of acetonitrile + 0.1% methanoic acid) and equilibrated (95% acetonitrile + 0.1% methanoic acid). Fractions of purified peptides were collected after sequential elution with 1 mL of 100%, 90%, 80%, 70%, 60%, 50% and 0% acetonitrile added with 0.1% methanoic acid, freeze-dried and stored at -20 °C.

Fractionation of gac seed peptides

According to the DPPIV inhibitory assay, the HILIC 70% acetonitrile + 0.1% formic acid fraction was the best among the eight fractions. Hence, it was further fractionated using RP-HPLC. After dissolving in 5% acetonitrile + 0.1% methanoic acid, 1 mg of the sample was fractionated using RP-HPLC (Chromaster, Hitachi High-Tech; Tokyo, Japan) attached with a C18 column (4.6 mm × 250 mm; particle size 5 µm; Phenomenex; Torrance, CA, USA). Eluted fractions were monitored with an ultraviolet detector at a wavelength of 214 nm. The mobile phase was composed of solvent A (5% acetonitrile + 0.1% trifluoroacetic acid) and solvent B (95% acetonitrile + 0.1% trifluoroacetic acid) and the gradient was set for 90 min at a flow rate of 1 mL/min. The gradient was arranged as: 1) linear gradient from 0% to 20% solvent B (0–45 min); 2) linear gradient from 20% to 80% solvent B (45–85 min); and 3) isocratic elution maintained at 80% solvent B (85–90 min) before returning to the initial condition. Selected peptide fractions were lyophilized and stored at -20 °C.

Dipeptidyl peptidase IV inhibitory assay and half maximal inhibitory concentration determination

The DPPIV inhibitory activity assay of the gac seed peptides hydrolysate was based on the slightly modified and adapted method of Lacroix and Li-Chan (2012). Briefly, 25 µL of 2 mM NaOH was mixed with 25 µL of 1.6 mM Gly-Pro-p-nitroanilide and incubated at 37 °C for 10 min. 1% dimethyl sulfoxide (25 µL) served as a blank and the negative control solution while 25 µL of linagliptin (50 mM) served as the positive control. The sample (25 µL), blank, negative control and positive control were each mixed with 25 µL of DPPIV buffer (45 mM Tris-HCl pH 8.0; 125 mM NaCl + 2.4 mM KCl; 3 mM DTT) and incubated at 37 °C for 5 min. Then, 50 µL of DPPIV (1 U/µL) were added and allowed to react at 37 °C for 1 h after which it was stopped with 100 µL of 1 M sodium acetate (pH 4.0). Samples were analyzed using a SpectraMax 190 Microplate Reader (SpectraMax 190; Molecular Devices LLC; San José, CA, USA) at 405 nM absorbance and the percentage of DPPIV inhibitory activity (n = 3) was evaluated based on Equation 1:

$$\text{DPPIV inhibitory activity (\%)} = \{[(I_{control}-I_{blank})-(I_{sample}-I_{blank})] / (I_{control}-I_{blank})\} \times 100 \quad (1)$$

where $I_{control}$ is the inhibitory activity of the control, I_{blank} is the inhibitory activity of the blank and I_{sample} is the inhibitory activity of the sample.

The half maximal inhibitory concentration (IC_{50}) value of the peptides was calculated in triplicate using non-linear regression of the %DPPIV inhibitory activity from nine different concentrations (the sample concentration at 1 mg/mL, 0.5 mg/mL, 0.4 mg/mL, 0.3 mg/mL, 0.2 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL and 0.0125 mg/mL).

Identification and analysis of gac seed peptides sequence

The peptide sequence from the most potent fraction against DPPIV was determined using liquid chromatography-mass spectrometry. The samples were separated in an Ultimate 300 RSLC system (Ultimate, series300 RSCL; Dionex; Sunnyvale, CA, USA) coupled with a C_{18} column (75 µM × 150 mm; Thermo Scientific Inc.; Waltham, MA, USA) and analyzed using a Thermo Q-Exactive™ Mass Spectrometer (Thermo Q-Exactive™ Mass; Thermo Scientific Inc.; Waltham, MA, USA). The flow rate of the mobile phase, composed of solvent A (0.1% methanoic acid in water) and solvent B (0.1% methanoic acid in 95% acetonitrile), was set at 250 µL/min. The gradient program was performed in the following order: 1) 0–5.5 min, isocratic flow at 1% solvent B; 2) 5.5–39.5 min, linear gradient from 1% to 60% solvent B; 3) 39.5–49.4 min, from 60% to 80% solvent B; and 4) 49.5–60.0 min, gradient elution from 80% to 1% solvent B. A full mass spectrometry scan was carried out in the 100–2,000 m/z range and the 10 ions of the highest intensities were selected for tandem mass spectrometry scans. The LC-MS/MS raw data were analyzed using the PEAKS Studio X software (Bioinformatics Solutions Inc.; Waterloo, ON, Canada). The parameters were set as: 1) Open project structure: added data; 2) Enzyme: GI (combination of three or more enzymes); 3) Type of enzyme: unspecified; 4) Instrument: Orbitrap; and 5) de novo score: > 70%. The sequence identified through the database was manually validated by matching it against the tandem mass spectrometry spectrum in the raw data.

Gac seed peptide-dipeptidyl peptidase IV docking

The RCSB Protein Data Bank was used as the retrieval source for the crystal structure of the human DPPIV complex with the diprotin A inhibitor (entry code: 1WCY). Before docking, the bound diprotin A inhibitor was removed from the active site of the DPPIV model. The 3D structure of the purified peptides was sketched using the Accelrys Discovery Studio software (Accerlys software studio, Visualizer 3.0; Accelrys Software Inc.; BIOVIA, San Diego, CA, USA), with the purified gac seed peptides docked into the DPPIV binding site using the CDOCKER component and the energy was minimized using the CHARMM program. The docking result was based on the combined free energy scores of each model.

Statistical analyses

The SPSS software (version 17; Chicago, IL, USA) was used. Data were subjected to analysis of variance followed by mean comparisons based on Duncan's multiple range test ($p < 0.05$). Each result was presented as mean ± SD.

Results and Discussion

Characterization of dipeptidyl peptidase IV inhibitory activity of gac seed protein hydrolysate

The IC_{50} value (1,587 ± 0.9 µg/mL) of the GSP hydrolysate was calculated at different concentrations, as shown in

Fig. 1. Hatanaka et al. (2012) found that the DPPIV inhibitory activity of defatted rice bran peptides increased at higher concentrations of peptide hydrolysate. Establishing the *in vivo* concentration of peptides for significant inhibition of DPPIV activity is necessary to achieve long-term beneficial effects (Velarde-Salcedo et al., 2013). It has been reported that the antidiabetic activity of bioactive peptides from several food sources inhibited carbohydrate-digesting enzymes and DPPIV, thereby enhancing insulin secretion, controlling satiety and reducing glucose absorption from the gut (Yan et al., 2019).

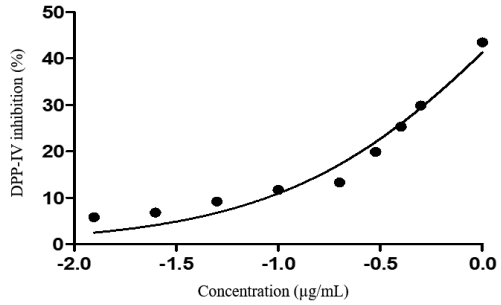

Fig. 1 Half maximal inhibitory concentration value of gac seed protein (GSP) hydrolysate (MW < 3 kDa), where x-axis = log concentration of GSP hydrolysate and y-axis = %dipeptidyl peptidase IV (DPPIV) inhibitory activity

Dipeptidyl peptidase IV inhibitory activity of hydrophilic interaction liquid chromatography-purified gac seed protein hydrolysate

HILIC, also considered a variant of normal phase liquid chromatography, separates polar compounds using a hydrophilic stationary phase and a hydrophobic organic mobile phase (Alpert and Andrews, 1988; Buszewski and Noga, 2012). Eight different peptide fractions were obtained from GSP hydrolysate through HILIC purification in this study. Their DPPIV inhibitory activity levels (1 mg/mL) were tested using *in vitro* DPPIV inhibitory assay (Fig. 2A). The HILIC fraction of 70% ACN + 0.1% FA had the highest DPPIV inhibitory activity (49.26 ± 0.63%) with an estimated IC_{50} value of 1,071 ± 0.7 µg/mL (Fig. 2B). This HILIC fraction contained predominantly organic solvent (70% ACN containing 0.1% FA), leading to less isolation of polar peptides than for the other fractions, implying the possible co-existence of both hydrophobic and hydrophilic peptides in it. This was also supported by the increased retention time in HILIC with the polarity (hydrophilic) of the peptide. HILIC elutes polar analytes through an increasing polar elution gradient (Cubbon et al., 2007). Furthermore, given that Nongonierma and FitzGerald (2013) reported high DPPIV inhibitory peptides contain higher numbers of hydrophobic amino acids, the HILIC fraction of 70% ACN containing 0.1% FA was submitted to further purification, as it may potentially contain DPPIV inhibitory peptides.

Dipeptidyl peptidase IV inhibitory activity of hydrophilic interaction liquid chromatography 70% acetonitrile + 0.1% methanoic acid fractions derived through RP-HPLC

Further separation of the most potent HILIC fraction (HILIC 70% acetonitrile containing 0.1% FA) with RP-HPLC produced 14 sub-fractions (F1-F14) (Fig. 3A). These fractions were collected individually and their DPPIV inhibitory activities were determined (Fig. 3B). Fractions F1 to F14 gave DPPIV inhibitory activities of 8.54±0.40, 6.34±0.14, 1.58±0.14, 4.67±0.27, 1.58±0.27, 12.92±0.54, 11.88±0.40, 13.87±0.48, 11.25±0.28, 9.03±0.25, 6.58±0.49, 9.98±0.22, 12.92±0.48 and 8.79±0.41%, respectively. The highest DPPIV inhibitory fractions (F6, F8 and F13) were chosen for identification of potential DPPIV inhibitory peptides.

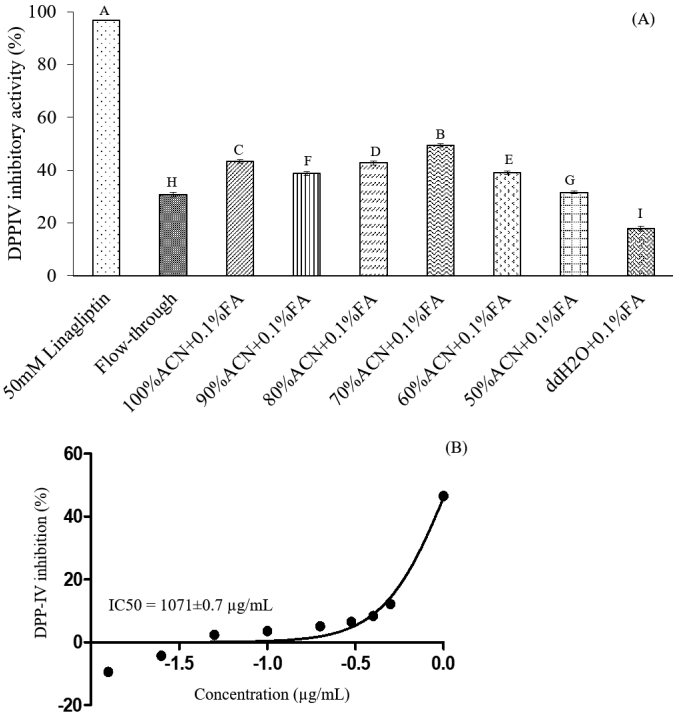

Fig. 2 (A) Inhibition of dipeptidyl peptidase IV (DPPIV) using different GSP hydrolysate-derived hydrophilic interaction liquid chromatography (HILIC) fractions (MW < 3 kDa), where different capital letters above bars indicate significant ($p < 0.05$) differences between the groups and error bars represent ±SD; (B) half maximal inhibitory concentration value of HILIC 70% acetonitrile + 0.1% methanoic acid, where x-axis = log concentration of HILIC 70% acetonitrile+0.1% methanoic acid (FA) and y-axis = % DPPIV inhibitory activity

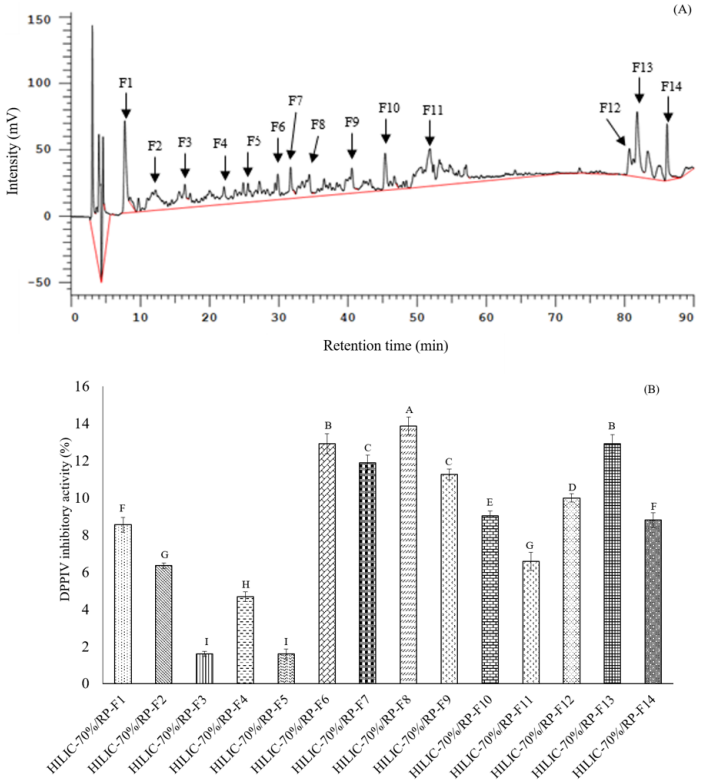

Fig. 3 (A) Reversed-phase high-performance liquid chromatography (RP-HPLC) chromatogram of hydrophilic interaction liquid chromatography (HILIC) 70% acetonitrile + 0.1% methanoic acid fractions; (B) dipeptidyl peptidase IV (DPPIV) inhibitory activities of 14 fractions of HILIC-70% acetonitrile + 0.1% methanoic acid separated using RP-HPLC, where x-axis = 70% ACN+0.1% FA of each fraction (F1–F14) and different capital letters above bars indicate significant ($p < 0.05$) differences between the groups and error bars represent ±SD.

Liquid chromatography-tandem mass spectrometry identification of peptides in hydrophilic interaction liquid chromatography-70 sub-fractions 6, 8, 13

The HILIC-70/RP-F6, HILIC-70/RP-F8 and HILIC-70/RP-F13 fractions had high DPPIV inhibitory activity and possibly contained potent DPPIV inhibitory peptides that were identified using LC-MS/MS. The lack of a completely decoded gac fruit genome led to non-comprehensive peptide identification through database-assisted sequencing. Therefore, LC-MS/MS and de novo sequencing using the PEAK Studio 8.0 software was implemented for peptide identification (Table 1). Of the total of 10 peptides, 2 were identified in HILIC-70/RP-F6, 2 in HILIC-70/RP-F8 and 6 in HILIC-70/RP-F13 fractions. To determine the highest potent DPPIV inhibitory peptides, their bioactivities were predicted using the BIOPEP database according to the methods of Mune et al. (2018) and Szerszunowicz and Nalecz (2018). The peptides with the top three highest BIOPEP scores—MAVA (0.00148), MAGL (9.56E-05), and DGSL (9.93E-05)—were selected as the DPPIV inhibitory peptide candidates. It has been reported that peptides that showed high DPPIV inhibitory activities typically contained a high level of hydrophobic amino acids (Nongonierma and FitzGerald, 2019). In addition, Arulmozhiraja et al. (2016) reported that hydrophobic pockets in the active sites of DPPIV may play a vital role in the inhibition of enzymes by peptides. The MS/MS spectra of MAVA (m/z 391.20), MAGL (m/z 391.19), and DSGL (m/z 391.17) peptides are shown in Fig 4 A–C, respectively.

Table 1 Identification and bioactivity of peptides from hydrophilic interaction liquid chromatography (HILIC)-70/RP-F6, HILIC-70/RP-F8 and HILIC-70/RP-F13

Fraction sample	Sequence	Mass (m/z)	RT (min)	Score	BIOPEP[a]	Bioactivity
HILIC-70/RP-F6	GGEE	391.1489	11.43	578	0	-
	MAGL	391.2015	45.21	2176	9.56E-05	DPPIV-inhibitory
HILIC-70/RP-F8	AEVGQCAVF	923.4271	14.07	708	0	-
	LTSA	391.2219	58.13	2756	0	-
HILIC-70/RP-F13	DGSL	391.1785	12.53	630	9.93E-5	DPPIV-inhibitory
	MAVA	391.2010	31.03	1504	0.00148	DPPIV-inhibitory
	LTSA	391.2222	30.51	1480	0	-
	LGMA	391.2047	27.20	1326	0	-

RT = Retention time

[a] BIOPEP database; http://www.uwm.edu.pl/biochemia/index.php/pl/biopep

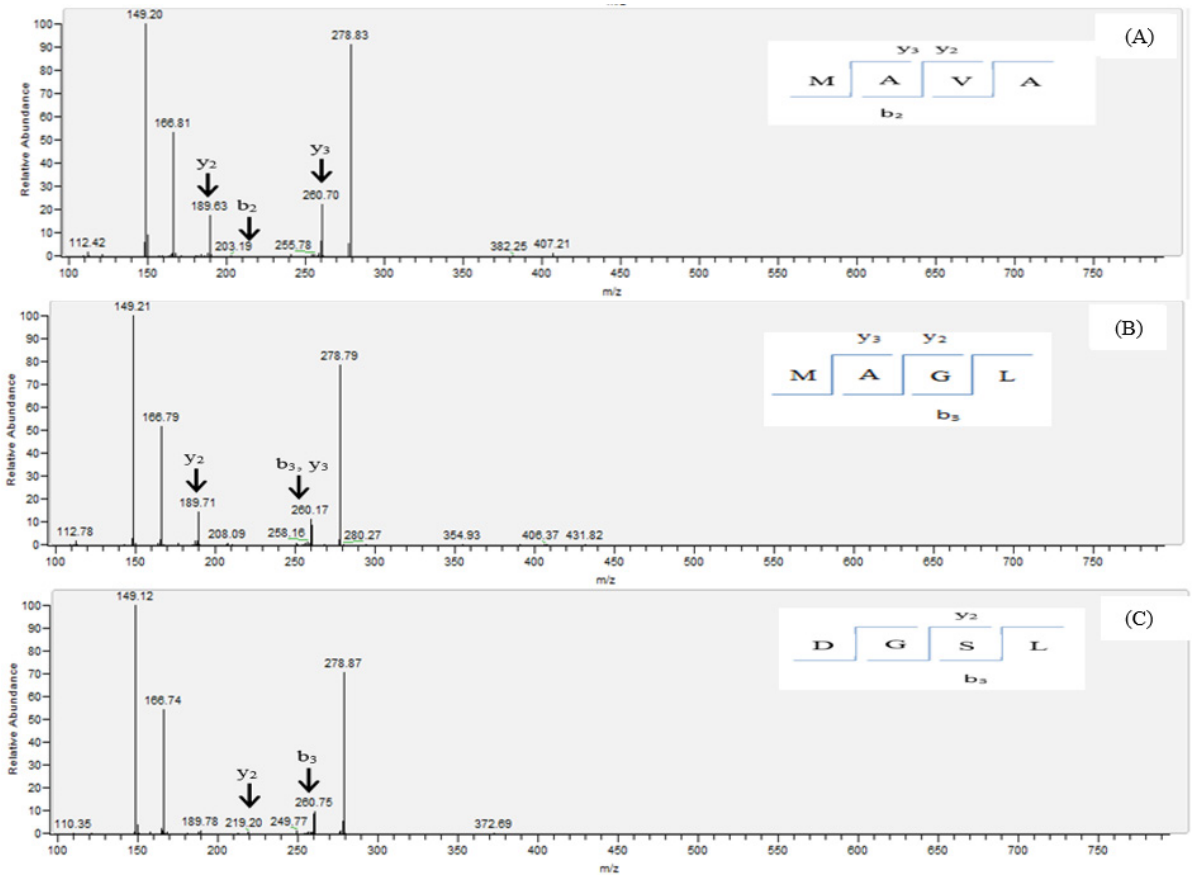

Fig. 4 Tandem mass spectrometry (MS/MS) spectra of potent dipeptidyl peptidase IV-inhibitory peptides from hydrophilic interaction liquid chromatography (HILIC)-70/RP-F6 and HILIC-70/RP-F13: (A) MAVA (m/z 391.2010); (B) MAGL (m/z 391.2015); (C) DGSL (m/z 391.1785), where MS/MS data were acquired under positive mode, MS/MS fragments of each peptide are denoted as b series ions and y series ions, x-axis = m/z (mass-to charge ratio) of dipeptidyl peptidase IV-inhibitory peptides from each fraction and y axis = % relative abundance (relative intensity) of a mass spectrum

Molecular docking of peptide sequences at the dipeptidyl peptidase IV binding site

Out of the 10 peptide sequences identified, MAVA, MAGL and DGSL docked within the DPPIV binding. MAVA and MAGL interacted with DPPIV through two H-bonds at Glu205 and Glu206 residues (Figs. 5A and 5B), while the peptide DGSL formed three H-bonds at Glu205, Ser630 and His740 (Fig. 5C). Furthermore, MAVA exhibited six van der Waals interactions with DPPIV at Glu205, Glu206, Tyr662, Tyr547, Tyr666 and Phe357, while MAGL had four van der Waals interactions at Lys554, Tyr547, Glu205 and Glu206, with DSGL having four van der Waals interactions at Glu205, Ser630, His740 and Arg125. Juillerat-Jeanneret (2014) recorded two pockets (S1 and S2) within the DPPIV active site; the S1 pocket had key residues as Tyr631, Val656, Trp659, Tyr662 and Val711, while the S2 pocket had key residues as Arg125, Glu205, Glu206, Phe357, Ser209 and Arg358. Furthermore, it has been reported that DPP-IV has three (instead of two) active sites (S1, S2 and S3), where S1 consists of strong hydrophobic interacting residues, such as Tyr547, Ser630, Tyr631, Val656, Trp654, Tyr662, Tyr666, Asn710, Val711 and His740. Interestingly, Ser630, Asn710 and His740 form a catalytic triad. S2 is seen as the socket near Glu205, Glu206 and Tyr662, while S3 consists of Ser209, Arg358 and Phe357 (Fan et al., 2013). Interactions between MAVA, MAGL and DGSL with DPPIV are emphasized in Table 2. DGSL had the lowest CDOCKER interaction energy (-67.3806 KJ/mol) and formed interactions with Glu205 and Arg125 as a binding in the S2 pocket, and interactions with Ser630 and His740 in the S1 pocket. Ser630 and His740 being part of the catalytic triad, promoted easier interactions. The CDOCKER interaction

energy of MAGL was -60.1354 KJ/mol and formed interactions with Glu205 and Glu206 in the S2 pocket and with Tyr 547 in the S1 pocket. MAVA formed interactions with Tyr662, Tyr547 and Tyr666 as a binding in the S1 pocket, and with Glu205, Glu206 and Phe357 in the S2 pocket with a CDOCKER interaction energy of -59.1096 KJ/mol. The docked conformations of the peptide ligands in increasing energy order were DGSL, MAGL and MAVA.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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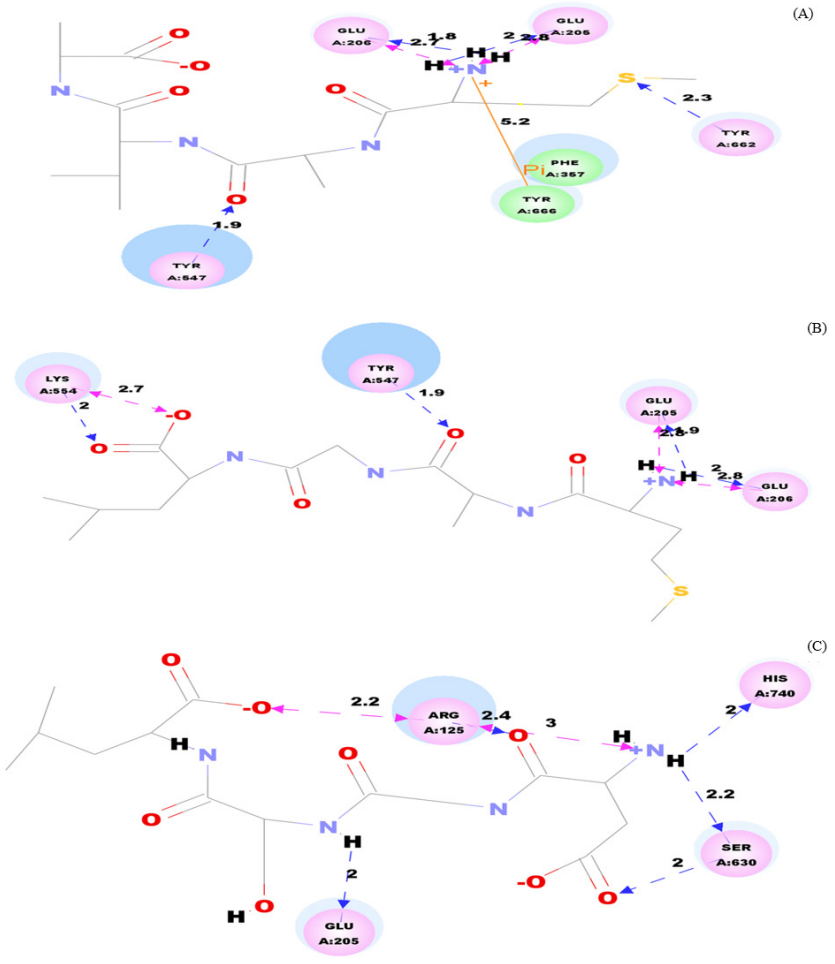

Fig. 5 Molecular interactions of three peptides with dipeptidyl peptidase IV pockets: (A) MAVA; (B) MAGL; (C) DSGL

Table 2 Molecular docking of three peptides with dipeptidyl peptidase IV (DPPIV) pockets

Sequence	DPPIV pocket	CDOCKER interaction energy (KJ/mol)
MAVA	Tyr662, Glu205, Glu206, Phe357, Tyr666, Tyr547	-59.1096
MAGL	Glu205, Glu206, Tyr547	-60.1354
DGSL	Arg125, Glu205, Ser630, His740	-67.3806

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