

# DISTILLERS SOLUBLES FROM ONTARIO CORN ETHANOL INDUSTRIES AS A NOVEL BIORESOURCE OF ANTI-DPP4 PEPTIDES

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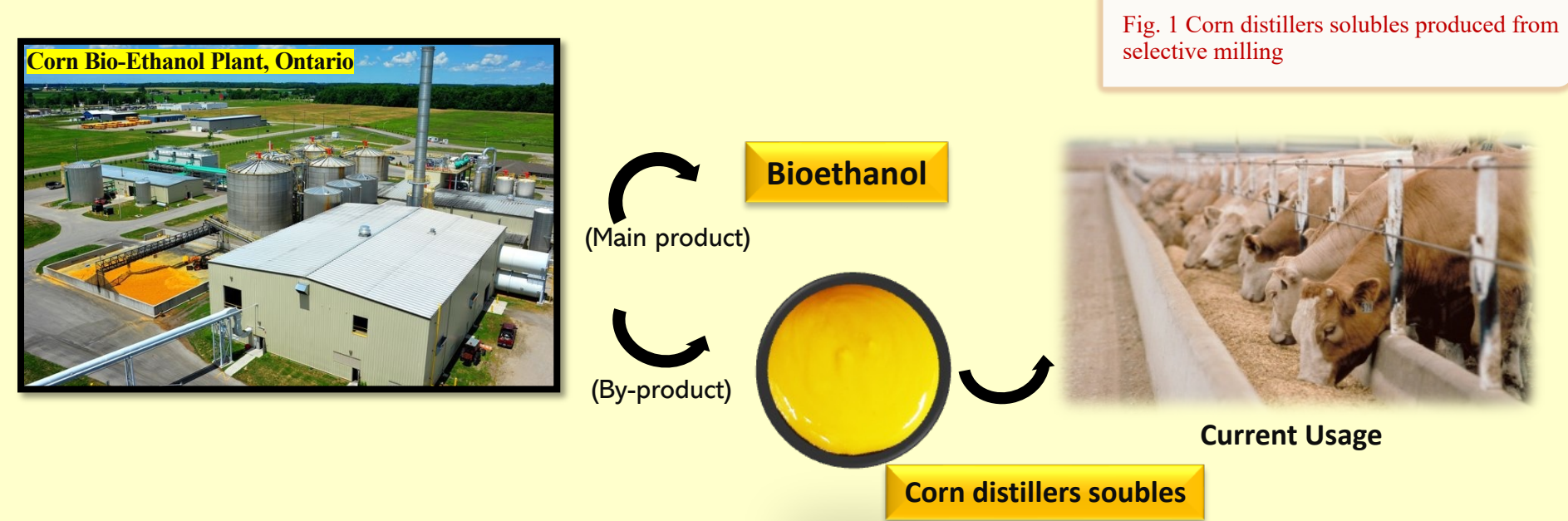


## ABSTRACT

- Protein concentrate was extracted from corn distillers solubles.
- Proline, glutamic acid, and leucine were the key amino acids of protein concentrate.
- Protein hydrolysates were prepared from protein concentrate by using four distinct enzymes among which alcalase was found to be the best choice for the hydrolysis.
- Optimal E:S ratios and incubation times providing maximum values of DPP4 inhibition for alcalase were 7.90% and 10.82 hrs.
- Yield and degree of hydrolysis for protein hydrolysate created by alcalase were maximum.
- The peptides, WNGPPGVF in PH-Ala, WPLPPFG in PH-Pap, LPPYLPS in PH-Pap, TPPFHLPPP in PH-Flav, and DPANLPWG in PH-Flav were found with greater inhibition potential against the DPP4 enzyme.
- The major interactions of the peptides with DPP4 enzyme were via hydrogen bond-, and hydrophobic interactions.
- The PHs can be used as ingredients in the feed or food industries with possible health advantages.

## INTRODUCTION

- In recent years, protein retrieval from agri-food industrial byproducts has gathered increased interest due to an increased demand for enhancing the sustainability of the agri-food chain.
- The exploration for protein hydrolysates (PHs) and peptides capable of inhibiting dipeptidyl peptidase (DPP4) enzyme has been increased due to higher demands for treating diabetes.
- The PHs can have probable applications in the industries like food, animal agriculture, aquaculture, nutraceuticals and/or pharmaceuticals (Zhu et al., 2019).
- In this scenario, corn bioethanol industries are becoming an important source of plant-based proteins.
- These industries' underutilized, inexpensive byproducts may offer a choice to comply with the burgeoning need for proteins.
- For example, IGPC Inc., an Ontario-based corn bioethanol company, generates a huge quantity (~16000 MT) of corn distillers solubles (CDS) annually using selective milling technology.
- The CDS is a low-value and underexplored by-product, with a protein content of 17–23% (dry basis) (Sharma et al., 2021).
- The CDS proteins have received less attention.



## OBJECTIVES

- To demonstrate the feasibility of expanding a value-chain for CDS proteins by enzymolysis, unraveling it as a source of "new and/or known peptides".
- To characterize and identify the proteins and peptides in PHs, anticipating their unknown bioactivities by BIOPEP tool.
- To simulating by molecular docking along with chemometric analysis of prime anti-DPP4 peptides.

## MATERIALS AND METHODS

- The buckets (20 L) containing CDS were brought from IGPC Ethanol Inc., Aylmer, Ontario that was stored for future experiments at -20°C.
- Alcalase® 2.4L, L 2.4 U/g, trypsin (1,000-2,000 units/mg dry solid), papain (1.5-10 units/mg solid), and flavourzyme, LE: U/g, ACE from rabbit lung (A6778), HHL (H1635), DPP4 IV human (D4943).

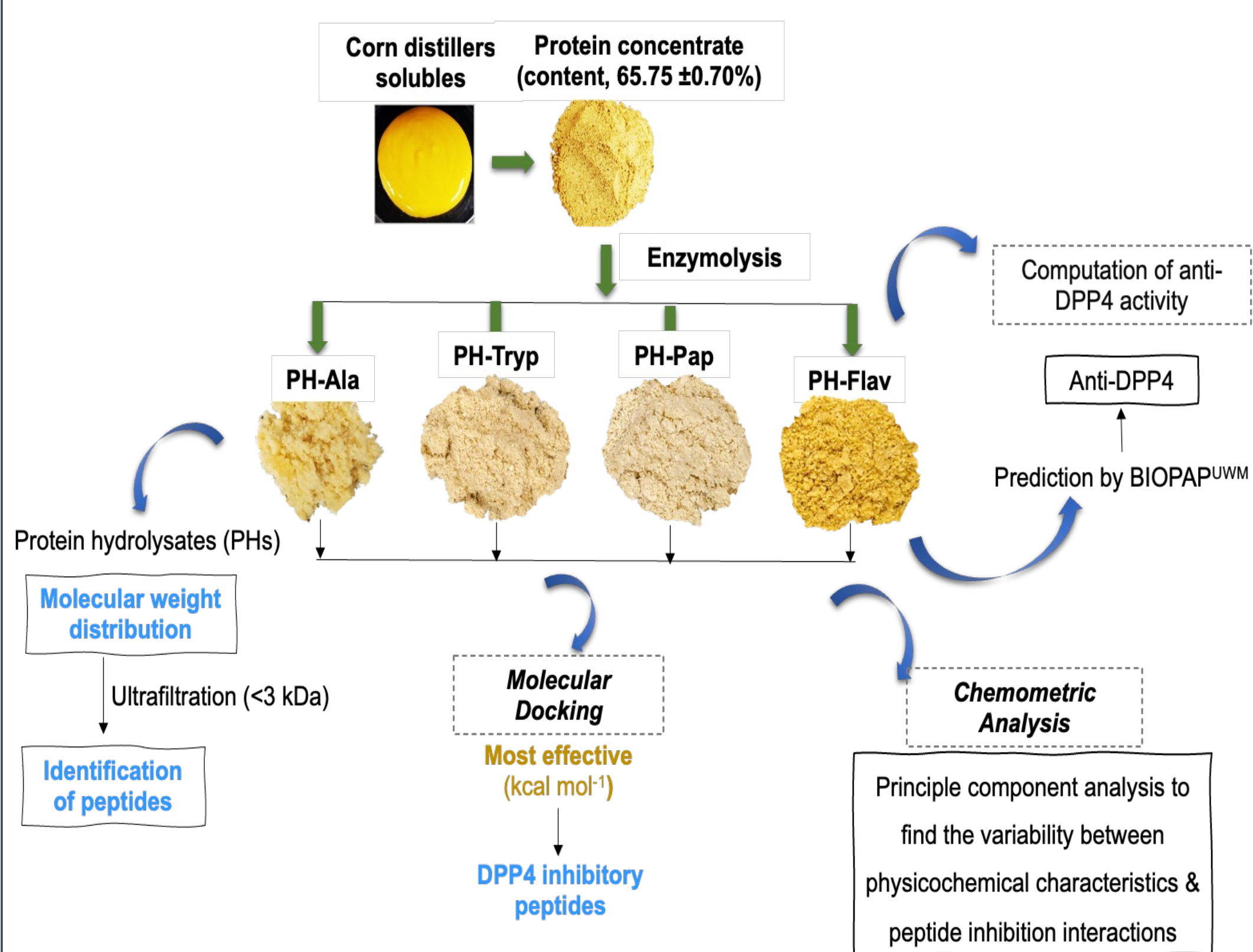


Fig. 2. Design for conducting experiment.

- Amino acids and solubility of protein concentrate and PHs were assessed (Sharma et al., 2022).
- Degree of hydrolysis was determined according to a previous method (Sharma et al., 2022).
- The PHs for their DPP4 inhibitory activity were assessed by following Amiri Sarteshnizi et al. (2021).
- The chances of peptides to be "bioactive" were examined with the PeptideRanker based on peptide scores ([http://bioware.ucd.ie/~compass/biowareweb/Server\\_pages/peptideranker.php](http://bioware.ucd.ie/~compass/biowareweb/Server_pages/peptideranker.php)).
- Peptides with scores > 0.50 were considered "bioactive" and greater scores mean higher bioactivity.
- BIOPEP (<http://www.uwm.edu.pl/biochemia/index.php/pl/biopep/>) and PepDraw (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>) for corresponding disparate bioactivities and peptides physicochemical characteristics were applied.

## RESULTS

### Optimization of protein hydrolysates for DPP4 inhibition activity

#### Alcalase hydrolysis:

$$\text{DPP4 inhibition (\%)} = 1.18A + 4.31B + 0.19AB - 0.13A^2 - 0.32B^2 + 7.91 \quad (1)$$

#### Papain hydrolysis:

$$\text{DPP4 inhibition (\%)} = 0.53A + 0.94B + 0.25AB + 0.02A^2 - 0.09B^2 + 17.22 \quad (2)$$

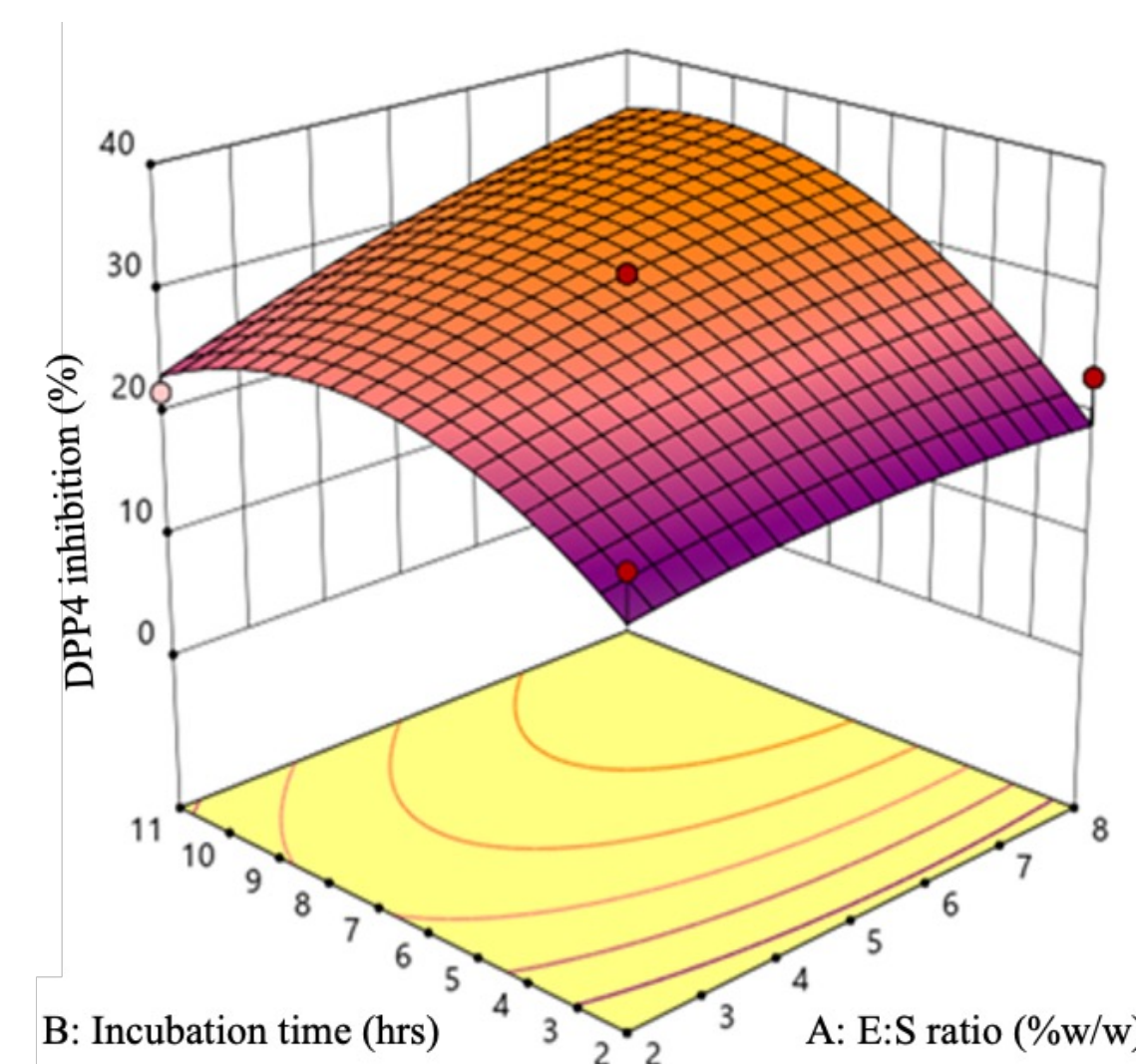
#### Trypsin hydrolysis:

$$\text{DPP4 inhibition (\%)} = 4.75A + 4.73B + 0.20AB - 0.35A^2 - 0.27B^2 - 9.93 \quad (3)$$

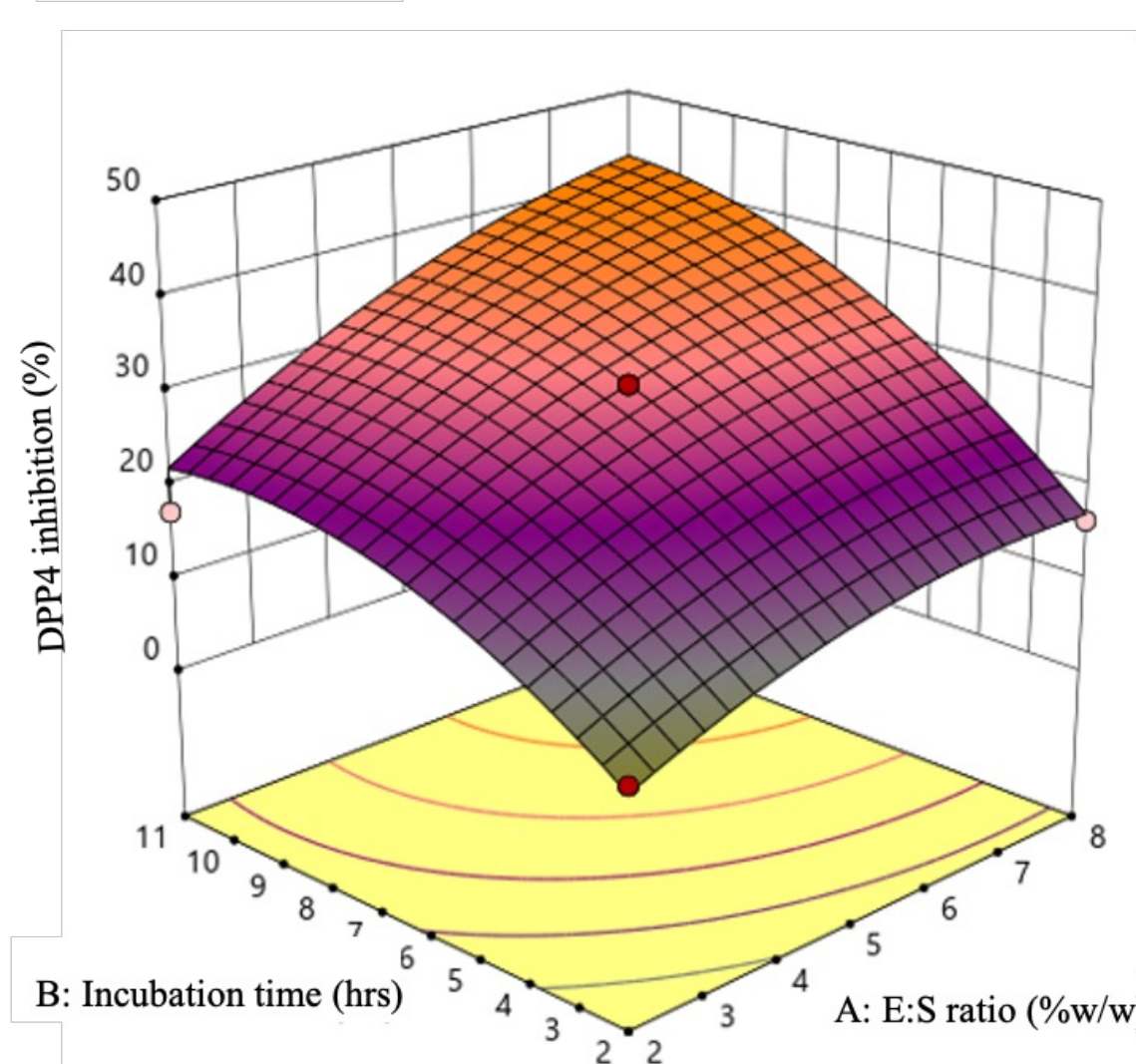
#### Flavourzyme hydrolysis:

$$\text{DPP4 inhibition (\%)} = 0.49A + 0.12B + 0.17AB + 0.05A^2 + 0.06B^2 + 2.03 \quad (4)$$

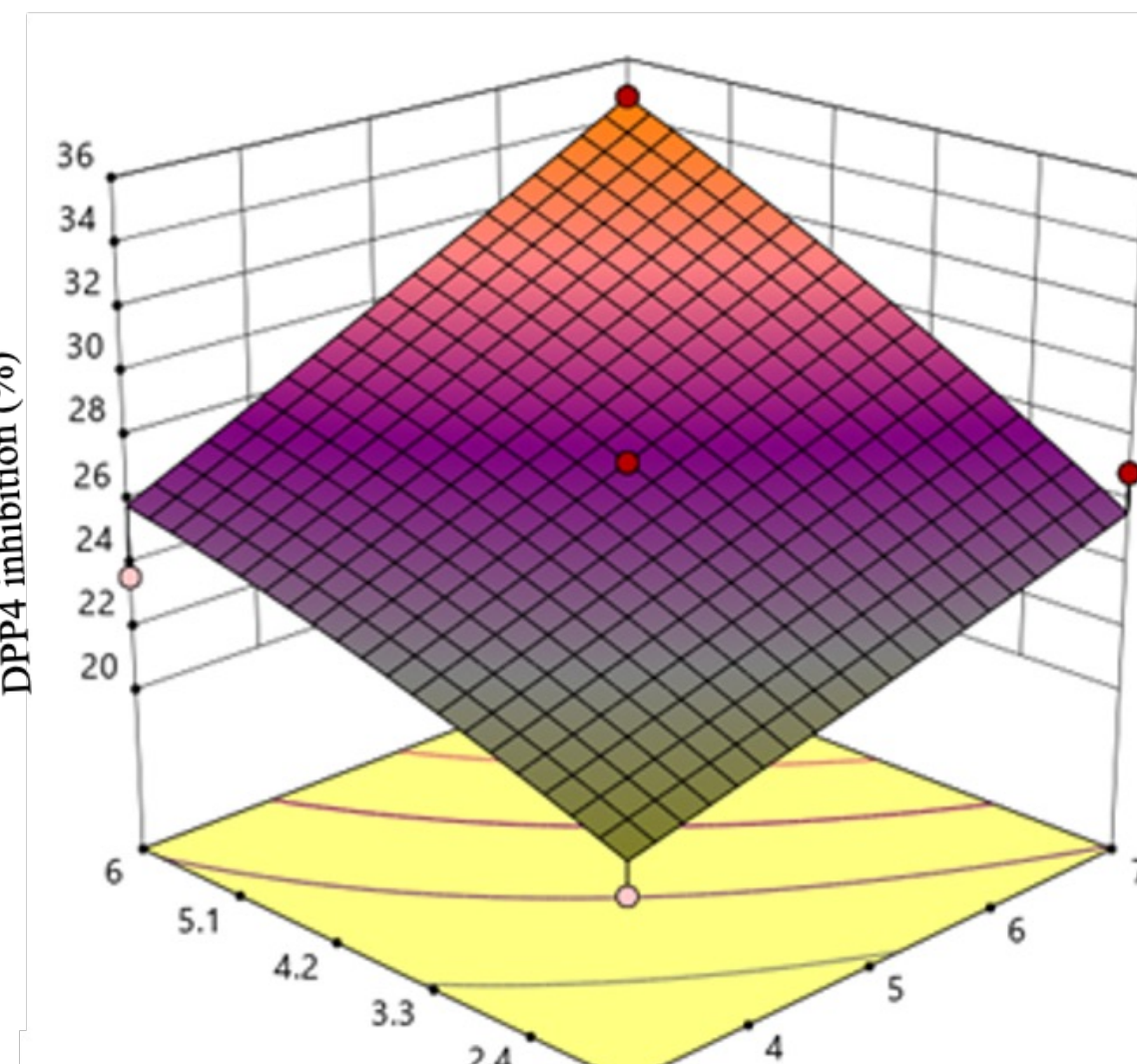
Where, A and B are enzymes to substrate ratio (%) and incubation time (hrs), respectively.



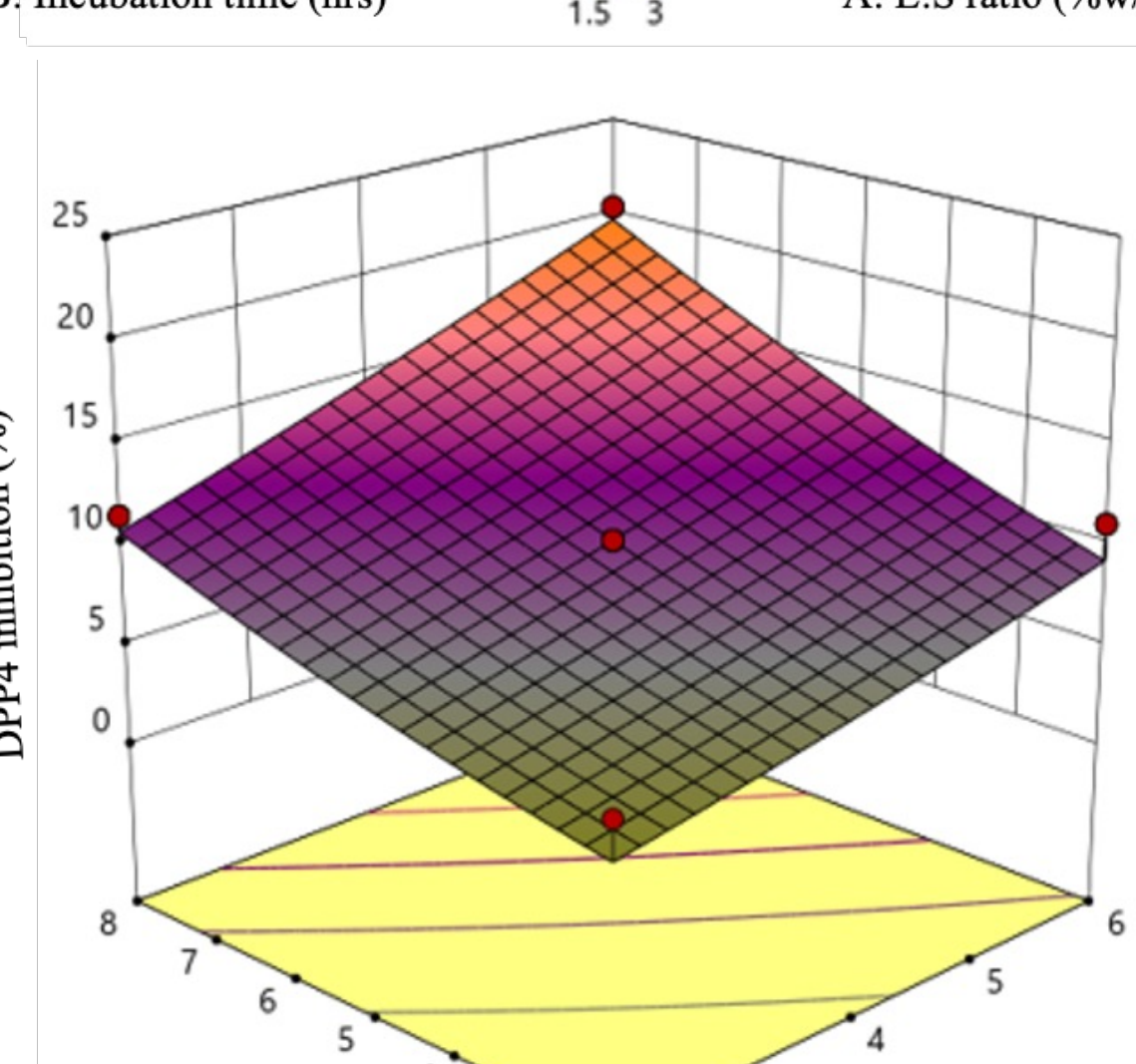
Alcalase



Trypsin



Papain



Flavourzyme

Fig. 3. Response surface graphs for maximizing the DPP4 inhibitory activity

- Optimal E:S ratios and incubation times providing maximum values of DPP4 inhibition for alcalase, papain, trypsin, and flavourzyme were (7.90%, 10.82 hrs), (7.0%, 6.0 hrs), (7.94%, 10.78 hrs), and (6.0%, 8.0 hrs), respectively.
- Optimized values of dependent parameters were tested by further experimentation.
- Predicted responses agreed with experimental values (relative error < 10%).

### Effect of ultrafiltration of protein hydrolysates on DPP4 inhibition activity

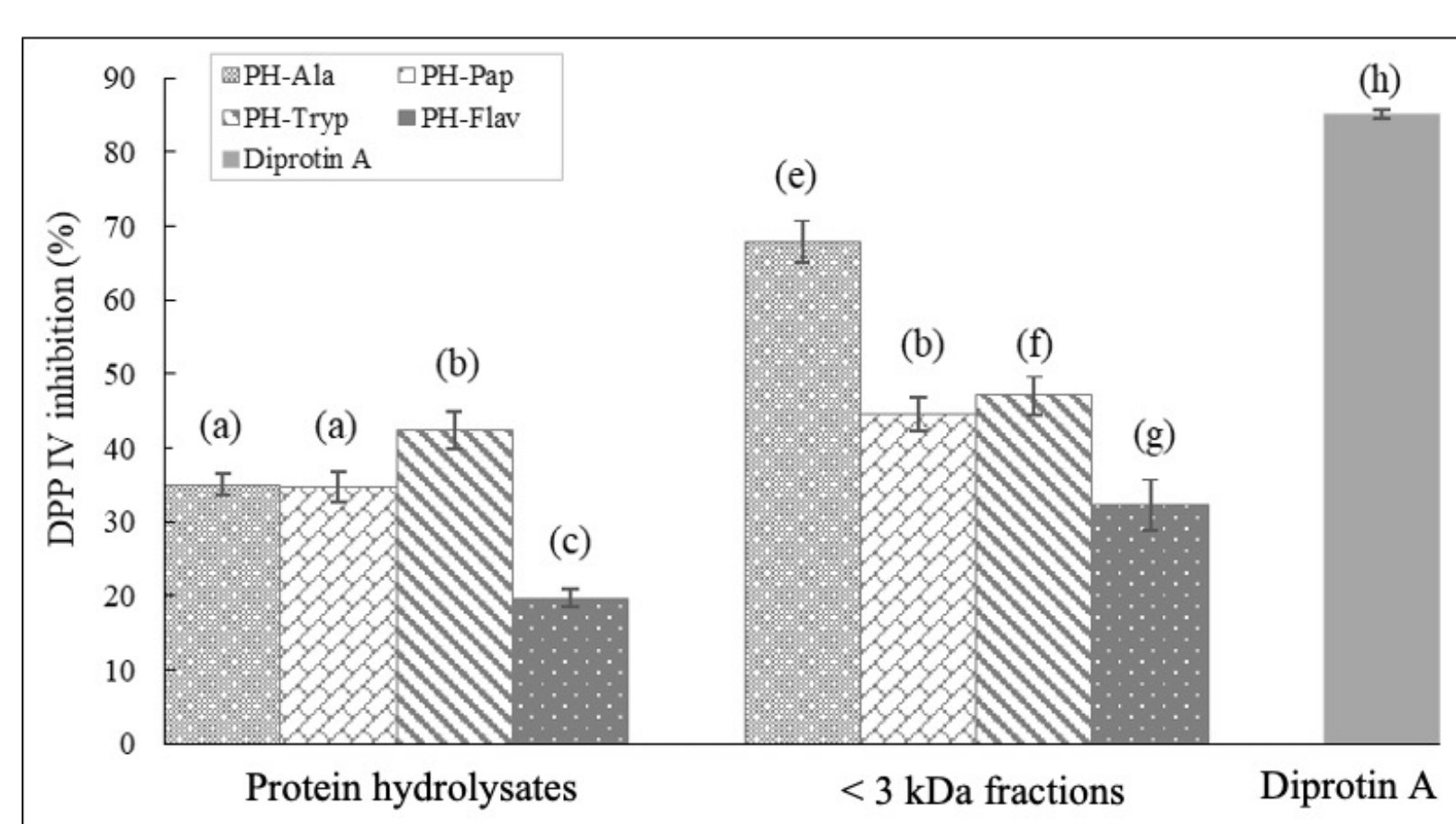


Fig. 4. DPP4 inhibition of protein hydrolysates and their purified fractions (<3 kDa) at 5.0 mg.mL<sup>-1</sup> from hydrolysis by alcalase, papain, trypsin, and flavourzyme. Different letters indicate statistically significant differences between samples (p < 05).

### Characterization of different protein hydrolysates

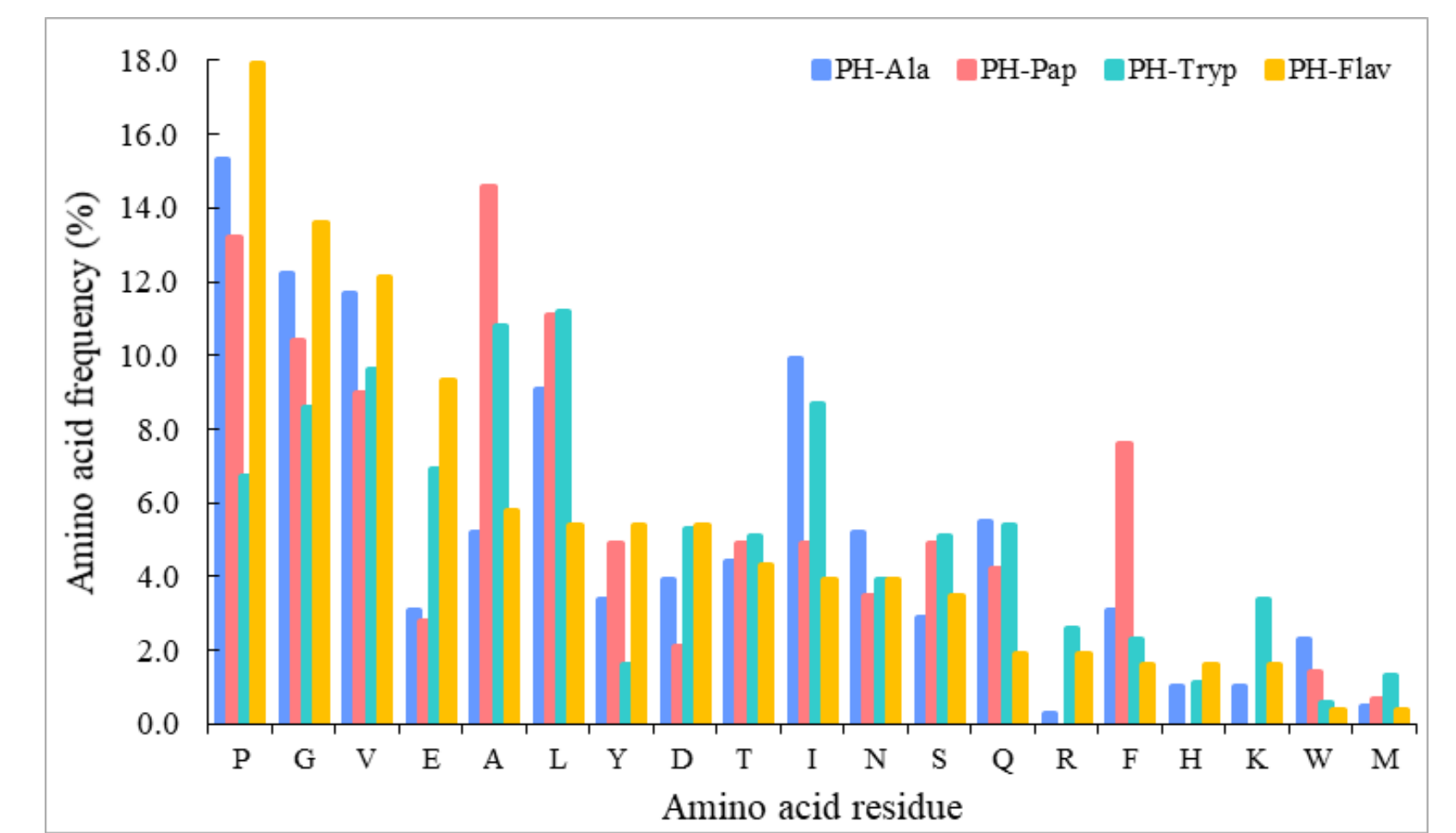


Fig. 5. Amino acid composition of PH-Ala, PH-Pap, PH-Tryp, PH-Flav built on the peptides characterized by mass spectrometry.

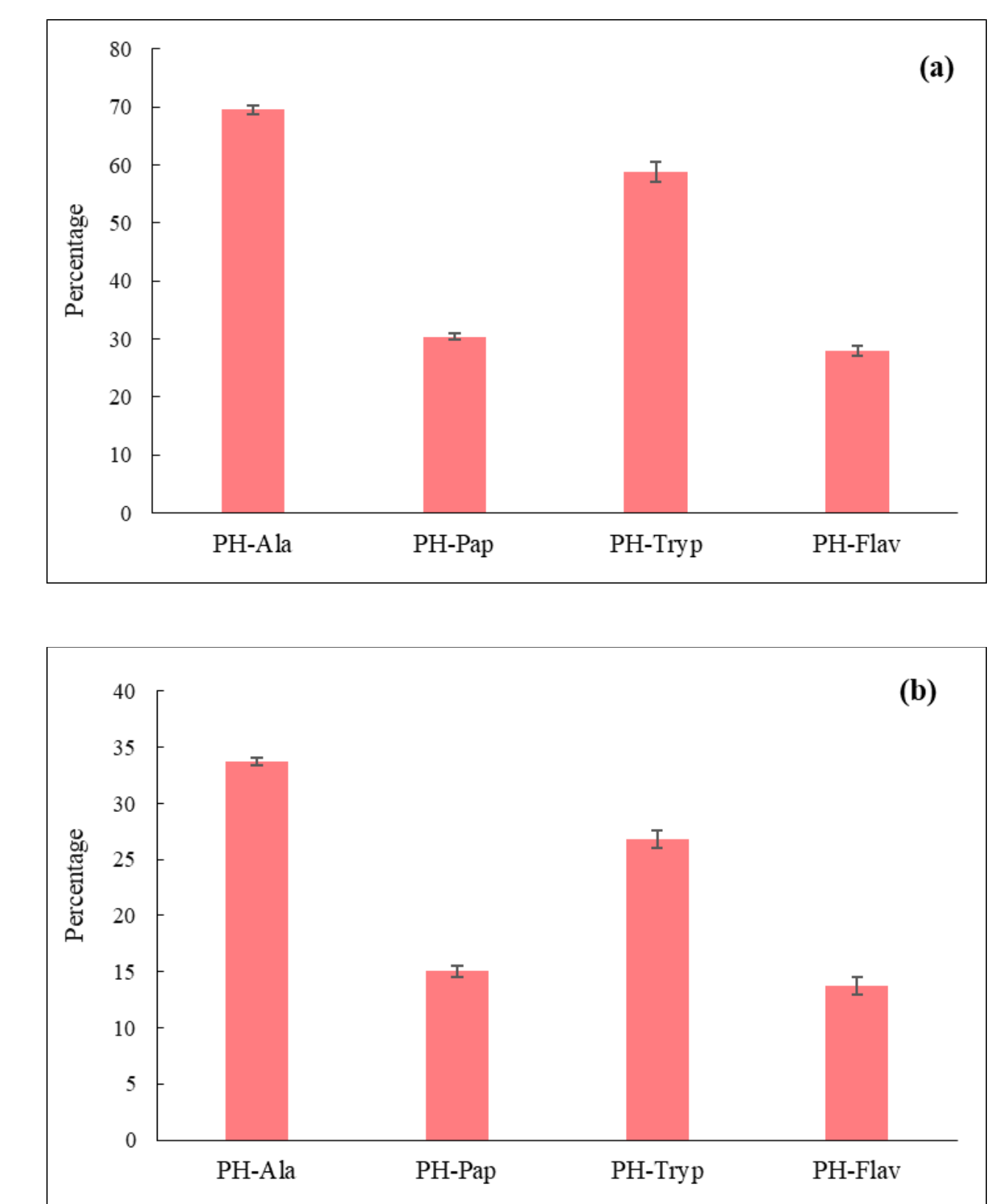


Fig. 6. Variations in the (a) yields, (b) degree of hydrolysis of different protein hydrolysate prepared by four different proteases.

### Analysis of identified peptides

Table 1. Design for conducting experiment.

MW	Peptides	pI	Hydrophobicity	Toxicity	Score	DPP4 inhibitory fragments
824.38	DWHTGPL	4.98	+12.07	NT	0.83	GP, PL, HT, TG, WH
938.42	NDWHTGPL	4.98	+12.92	NT	0.78	GP, PL, HT, TG, WH, ND
1091.6	DWYKGPSTLL	6.69	+10.58	NT	0.71	GP, LL, WY, KG, PT, TL, YK
839.47	SNIPLSPL	5.50	+6.33	NT	0.66	IP, SP, PL
808.50	GILPLSPL	5.58	+4.92	NT	0.64	LP, SP, LPL, PL, GI, IL
741.44	VLPVPAP	5.46	+4.80	NT	0.63	PA, LP, VP, AF, PV, VL
964.57	AIVLPPWVA	5.61	+3.80	NT	0.62	PP, VA, LP, WV, PW, VL
803.42	VGSPFLGQ	5.44	+8.15	NT	0.61	SP, FL, PF, VG
856.46	LPPYLSPA	5.58	+6.07	NT	0.55	PP, PA, LP, SP, PY, YL
801.40	NPALQPY	5.27	+8.34	NT	0.55	PA, NP, QP, AL, LQP, PY
843.45	NPILQPY	5.27	+6.72	NT	0.52	NP, QP, LQP, IL, PI, PY
874.45	YQQPIGG	5.50	+8.93	NT	0.52	GG, QP, II, PI, YQ, QQ
893.54	IVLPPWVA	5.60	+3.30	NT	0.50	PP, VA, LP, WV, PW, VL
872.42	WNGPPGVF	5.42	+7.07	NT	0.94	GP, PP, PPG, WN, GV, NG, PG, VF
1030.44	DFDFDIN	2.85	+10.63	NT	0.78	DN, IN
730.38	KNGGLGW	9.80	+11.66	NT	0.71	GL, GG, GW, NG
665.30	DLGGTGF	3.05	+12.28	NT	0.62	GG, TF
707.34	FIGDGS	3.12	+10.22	NT	0.55	SL
747.42	TIPTIGF	5.37	+5.74	NT	0.50	IP, GF, PT, TI

## CONCLUSIONS

- It is evident that alcalase is superior for PC in terms of yield (69.57±0.74%), degree of hydrolysis (33.76±0.34%), Mw (200-500, 500-800 Da), number of proteins (57), and bioactive peptides (19).
- The present findings revealed that the peptides were maximally associated with corn-derived zein and yeast-derived glyceraldehyde-3-phosphate dehydrogenase in all the PHs.
- The peptides impede tested enzymes majorly via hydrogen bond-, and hydrophobic interactions. For the DPP4 enzyme, prime bindings are with GLN553, GLU206, PHE364, VAL303, and THR304.
- The peptides, WNGPPGVF in PH-Ala, WPLPPFG in PH-Pap, LPPYLPS in PH-Pap, TPPFHLPPP in PH-Flav, and DPANLPWG in PH-Flav were found with greater inhibition potential against the DPP4 enzyme, verified by their lesser Ki values of 0.090, 0.064, 0.076, 0.039, and 0.039 μM, respectively.

## REFERENCES

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