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Purification of a novel monophenolase inhibitory peptides prepared from *Vicia faba* pods protein via enzymatic hydrolysis

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ABSTRACT

The aim of the present study was to prepare novel monophenolase inhibitory peptides from *Vicia faba* (broad bean) pods. Isolated protein was hydrolysed by immobilized protease at pH 10. For maximum production of hydrolysate (peptides), an optimized hydrolysis process, including incubation temperature and protein concentration, was established. Broad bean peptides had higher tyrosinase inhibitor potency than that of the parent protein. They were fractionated by ultrafiltration into three fractions: F_1, F_2, and F_3. With high monophenolase inhibitor potency, F_2 was further fractionated by reversed-phase high-performance liquid chromatography (RP-HPLC) three times, followed by high-performance size-exclusion chromatography (HPSEXC) to finally achieve a single peak, confirming its purity with a molecular weight of 26.102 kDa. It had superior monophenolase inhibitor potency compared to that of the original protein. The Michaelis-Menten constant (K_m) values of tyrosinase activity toward L-tyrosine in the presence of a broad bean monophenolase inhibitor increased when its concentration increased, while the maximum velocity (V_{max}) value was unchanged. The monophenolase inhibitor exhibited a competitive type of inhibition. The results of this study suggest that broad bean pods are a good source of monophenolase inhibitory peptides, which exhibit therapeutic potential for curing or preventing some diseases.

1. Introduction

Enzymatic production of bioactive peptides from food proteins has been previously identified. They act as antibacterial, anti-diabetic, antihyperlipidemic, antihypertensive, angiotensin I-converting enzyme inhibitors and antioxidants (Udenigwe and Aluko, 2012; de Castro and Sato, 2015; Corrons et al., 2017). They are also derived from marine sources (Slama et al., 2018). Protein hydrolysate with bioactive peptides can be considered as functional food. They are specific protein fragments that have functioning effects on living beings (Perez Espitia et al., 2012).

Tyrosinase (EC 1.14.18.1) is responsible for the formation of melanin in the hair, skin, and coloured part of the eyes. It catalyses the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), acting as monophenolase activity. Furthermore, it oxidizes L-DOPA to the corresponding o-dopaquinone, acting as diphenolase activity with formation of melanin (Mora and Baraldi, 2000). Excessive melanin formation, as a result of high tyrosinase level, causes skin hyperpigmentation diseases such as seborrheic keratosis, tinea versicolor, melasma, and malignant melanomas (Chang et al., 2013) and plays a role in neurodegenerative diseases such as Parkinson's disease. A high level of tyrosinase also leads to an increase in intracellular dopamine, which forms large amounts of melanin and causes cell death (Hasegawa, 2010). Oxidation of phenolic compounds by tyrosinase into quinine leads to browning of vegetables and fruits, which results in a loss of nutritional value.

Synthetic tyrosinase inhibitors, such as hydroquinone, kojic acid, and azelaic acid are used in the cosmetic industry as whitening agents (Nakayama et al., 2000). Benzoic acid (Khan et al., 2010), plant flavone quercetin (Taherkhani and Gheibi, 2014), essential oils from medicinal plants (Aumeeruddy-Elalfi et al., 2016), and some vegetables and fruits (Fawole et al., 2012) could be inhibited by tyrosinase activity.

The purpose of this study was to prepare tyrosinase (monophenolase) inhibitory peptides from broad bean pods. Broad bean protein was hydrolysed by immobilized lettuce protease. The hydrolysate was fractionated by ultrafiltration, and the most active fraction with high monophenolase inhibition activity was purified by reversed-phase-high-performance liquid chromatography (RP-HPLC). It was analysed by high-performance size-exclusion chromatography (HPSEXC), and the kinetics of the tyrosinase inhibitor were determined.

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Table 1
Tyrosinase (diphenolase and monophenolase) inhibitor potency of broad bean protein and its hydrolysate.

Samples	Yield	Tyrosinase inhibition activity			
		Diphenolase inhibitor potency		Monophenolase inhibitor potency	
	(μg/g dry pods)	(μg KE/μg)	Relative times	(μg KE/μg)	Relative times
Protein	76.28 ± 0.7	1.13 ± 0.0	1.0	0.56 ± 0.0	1.0
Hydrolysate	586 ± 38.2	1.81 ± 0.1**	1.6	0.80 ± 0.1*	1.6

Yield was expressed as μg/g dry broad bean pods.
Tyrosinase inhibitor potency was represent as μg kojic Equivalent per μg protein or peptides (μg KE/μg).
Relative potency was calculated as tyrosinase inhibitor potency of peptides per that of protein.
Data are expressed as mean ± SD; n = 3.
Mean assigned with * denotes a statistically significant difference at $p < 0.025$ when tyrosinase inhibition activity of hydrolysate was compared to that of protein.
Mean assigned with ** denotes a statistically significant difference at $p < 0.001$ when tyrosinase inhibition activity of hydrolysate was compared to that of protein.

Table 2
Effect of incubation temperatures and protein concentrations on peptides production from broad bean pods by immobilized lettuce protease.

Optimization of hydrolysis conditions		Peptides production	
		(μg/reaction mixture)	(μg/g dry pods)
Incubation temperature	70 °C	11.78 ± 0.64	586.8
	60 °C	6.48 ± 0.2*	323.04
road bean protein -concentration/R.M.	1.5 μg	11.78 ± 0.6	586.8
	3.0 μg	17.33 ± 0.6	863.3
	4.5 μg	23.44 ± 0.8	1167.6**
	6.0 μg	20.67 ± 0.5	1029.6

Peptides production was expressed as μg/g dry broad bean pods weight.
Data are expressed as mean ± SD; n = 3.
Mean assigned with * denotes a statistically significant difference at $p < 0.005$ when peptides production at 60 °C was compared to that at 70°c.
Maximum peptides production was significantly obtained at 4.5 μg/RM (**).

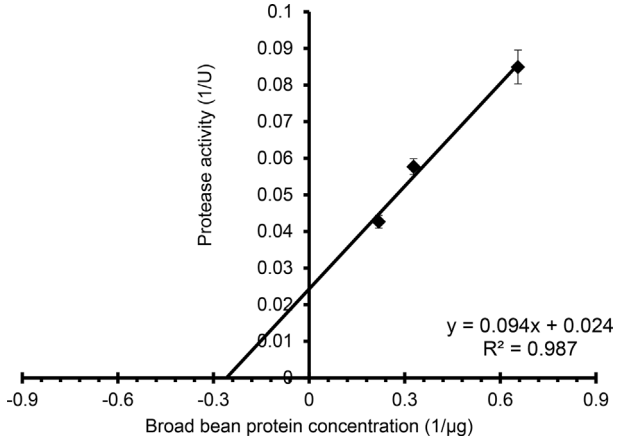


Fig. 1. Lineweaver-burk plot of immobilized lettuce protease towards broad bean protein. K_m = 3.92 μg and V_{max} = 41.61 U.

2. Materials and methods

2.1. Chemicals

L-Tyrosine, L-3,4-dihydroxyphenylalanine (L-DOPA), tyrosinase from mushroom, and kojic were obtained from Sigma chemical company (USA). Other chemicals and reagents used were of analytical grade.

2.2. Plant materials

Dry lettuce seeds (*Lactuca sativa*), family Asteracea, were bought from local markets (Cairo, Egypt). Fresh broad bean pods (Vicia faba), family Leguminoseae, were purchased from local markets (Cairo, Egypt).

2.3. Preparation of immobilized protease

Immobilized protease was prepared according to the method of Ali et al. (2016). Crude protease was extracted from dry lettuce seeds with 0.1 M Tris-HCl buffer, pH 10.0. Free protease was precipitated from the prepared crude enzyme by ammonium sulphate at 60% saturation. Alginate-glutraldehyde beads were prepared by dropping 2% sodium alginate into 0.2 M CaCl2 solution with continuous stirring and stored at 4 °C for 24 h prior to use. Alginate beads were activated by being added to 6.5% glutraldehyde in 0.2 M Tris-buffer at 25 °C with stirring for 2 h. Two g of calcium alginate activated beads were mixed with 1.5 mL of the free enzyme followed by adding 1.5 mL distilled water to ensure full immersion of beads in enzyme solution. The loading process was performed for 1 h under continuous shaking at 9 °C.

Table 3a
Diphenolase and monophenolase inhibitor potency of the ultrafiltration fractions.

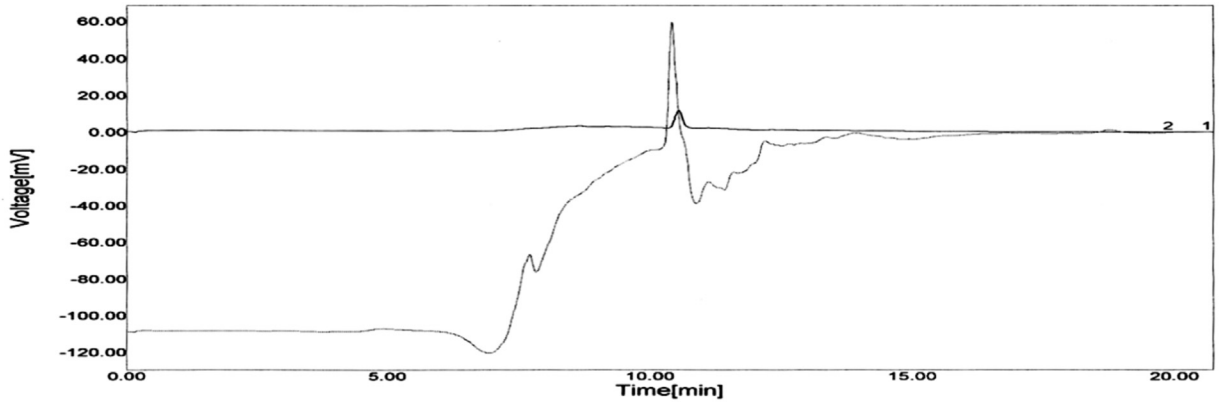
	Yield	Diphenolase inhibitor potency		Monophenolase inhibitor potency	
	(%)	(μgKE/μg)	Relative times	(μgkE/μg)	Relative times
Hydrolysate	100	1.81 ± 0.2	1.0	0.8 ± 1.2	1.0
F3	47.1	1.13 ± 0.0*	0.62	0.0	0.0
F2	14.0	0.0 ± 0.0	0.0	135.8 ± 9.0**	169.7
F1	38.90	408 ± 12.4**	225	111.4 ± 2.6**	139.2

Yield was expressed as % broad bean pods.
Tyrosinase inhibitor potency was represent as μg kojic Equivalent per μg protein or peptides (μg KE/μg).
Relative potency was calculated as tyrosinase inhibitor potency of peptides per that of protein.
Data are expressed as mean ± SD; n = 3.
Mean assigned with * denotes a statistically significant difference at $p < 0.005$ when tyrosinase inhibition activity of fraction was compared to that of hydrolysate.
Mean assigned with ** denotes a statistically significant difference at $p < 0.0005$ when tyrosinase inhibition activity of fraction was compared to that of hydrolysate.

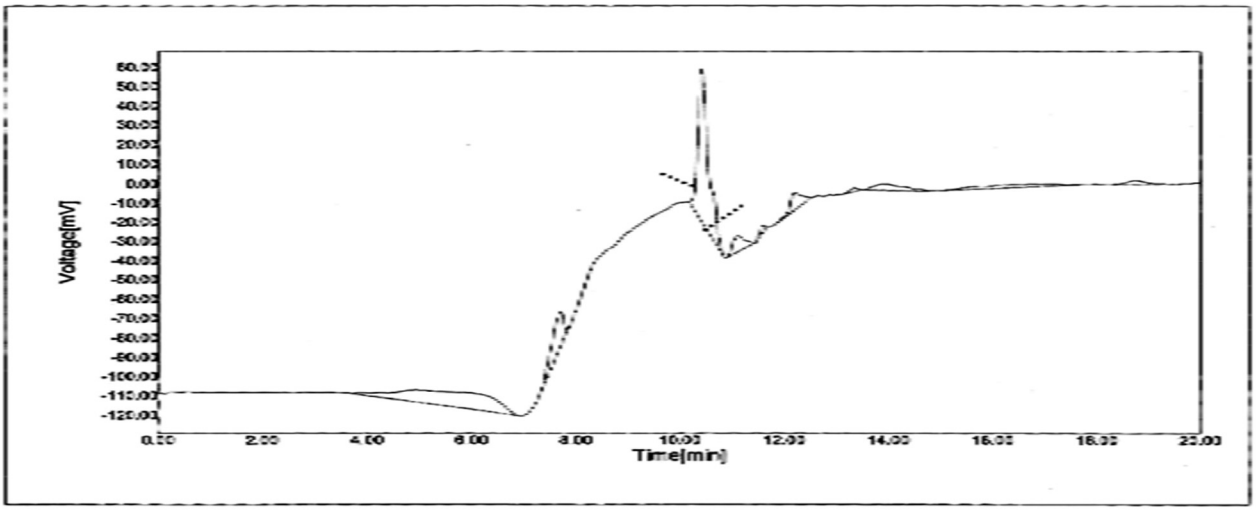
2.4. Extraction of broad bean protein

Broad bean pods were oven dried at 60 °C to inactivate the enzymes present. They were grinded and soaked with distilled water and left overnight at 4 °C. The homogenate was filtered by gauze followed by centrifugation at 3500 g for 10 min. The resulted supernatants were

First column



Second column.



Third column

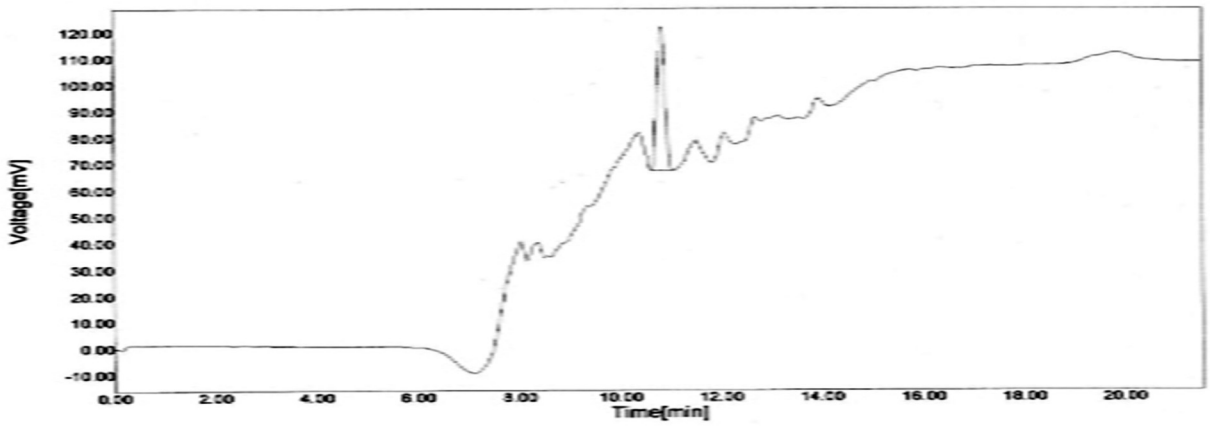


Fig. 2. Reversed-phase chromatographic separation of broad bean F_2. Absorbance was measured at 280 and 214 nm. Fractions were collected and assayed for tyrosinase inhibition activity. Only one fraction eluted at 10 min showed high monophenolase inhibition activity. It was selected for further fractionated by two successive RP-HPLC (second and third). Absorbance was measured at 214 nm.

Table 3b
Tyrosinase (monophenolase) inhibitor potency of HPLC collected peaks.

Samples	Relative peptides concentration	Monophenolase inhibitor potency	
	(%)	(μg KE/μg)	(Times)
F2	100	135.8 ± 9.0	1.0
Peak I	10.28	11275.7	83.0

Monophenolase inhibitor potency was represented as μg kojic Equivalent per μg peptides (μg KE/μg).

Table 4
HPSEX analysis of the standard proteins and tyrosinase (monophenolase) inhibitor.

No.	Retention time (min)	Matching standard	Molecular weight (KDa)
1	4.009	Tyrosine	0.181
2	3.687	Tripeptide (Hip-His-Leucine)	0.429
3	3.231	Lysozyme	14.307
4	3.232	Carbonic anhydrase	30.0
5	3.172	Broad bean monophenolase inhibitor	26.102

saturated with ammonium sulphate to a concentration of 70% and left overnight at 4 °C. The precipitates were collected by centrifugation at 3500 g for 10 min then dialyzed overnight against distilled water with dialysis bag molecular weight cut off 10–14 KDa. The supernatant was stored at −4 °C for further use as broad protein.

2.5. Preparation of broad bean peptides

Preparation of peptides was accomplished through enzymatic hydrolysis of broad protein. Immobilized protease beads were added to 0.1 mL of broad protein followed by 2 mL 50 mM Tris-HCl buffer, pH 10. The tubes were incubated for 1 h in a water bath at 70 °C. The reaction was stopped by decantation and the solution was collected and stored at −10 °C to be further used.

2.6. Determination of peptides concentration

Peptides concentration was estimated with phenol reagent (El-Sayed, 2001) using tyrosine as standard. To a mixture of 5 ml of the supernatant and 5 mL of 0.5 mL mM NaOH; 0.5 ml Folin reagent was added drop wise while shaking. After standing for 10 min at room temperature, the absorbance was measured at 660 nm. The concentration of hydrolysate was calculated as equivalent tyrosine (μg tyrosine).

2.7. Determination of optimum peptides production

The optimum enzymatic activity of immobilized lettuce protease towards casein was found previously at incubation temperature ranged from 60 to 70 °C and pH 10 (El-Sayed et al., 2018). Thus, the effect of incubation temperature 60 and 70 °C on peptides production from broad bean protein was studied. For maximum production of peptides, hydrolysis process at different broad bean protein concentrations was studied at 7 °C. Affinity of immobilized lettuce protease (apparent K_m) towards broad bean protein as substrate was investigated.

2.8. Fractionation of broad bean hydrolysate by ultrafiltration

Broad bean hydrolysate solution was successively ultrafiltrated. The ultrafiltration was performed using ultrafiltration centrifugal units (Amikon, Millipore, USA), with molecular weight cut-off 100 and 10 KDa. The centrifugation was first performed for molecular weight separation of 100 KDa cut-off centrifugal units at 5000 g for 30 min. Two fractions were collected, F3 with molecular weight above 100 KDa and F with molecular weight below 100 KDa. The last fraction F was ultrafiltered for molecular weight separation by 10 KDa cut-off centrifugal units at 7500 g for 15 min. Two fractions were collected F2 with molecular weight above 10 KDa and below 100 KDa and F1 with molecular weight below 10 KDa. Peptides concentration of each fraction was estimated. The three fractions collected F3, F2 and F1 were stored at −4 °C to be further used.

2.9. Reversed phase-high performance liquid chromatography (HPLC)

Fractions F2 from ultrafiltration with high monophenolase inhibitor potency were subjected to further purification using semi-preparative HPLC. The purification was performed on HPLC system model Waters 1500 with binary Pump Model 1525, using reversed phase C18 HypersilTM ODS 10 μm particle size 250 mm (Thermo, USA). The elution was performed using a primary wash run with flow rate 1 mL/min with a gradient from 100% solvent A (water) to 100% solvent B (60% methanol: 10% acetonitrile: 30% water with 0.1% TFA) from 0 to 5min then isocratic with 100% solvent B for 25 min. The second separation run was performed at flow rate 1 mL/min with a gradient from 100% solvent B (60% methanol: 10% acetonitrile: 30% water with 0.1% TFA) to 100% solvent C (100% methanol) from 0 to 6 min then isocratic with 100% solvent C till end of run at 30 min. Concentration of each peak was estimated. The peaks collected were dried and stored at −4 °C to be further used.

2.10. Determination of tyrosinase inhibitor potency

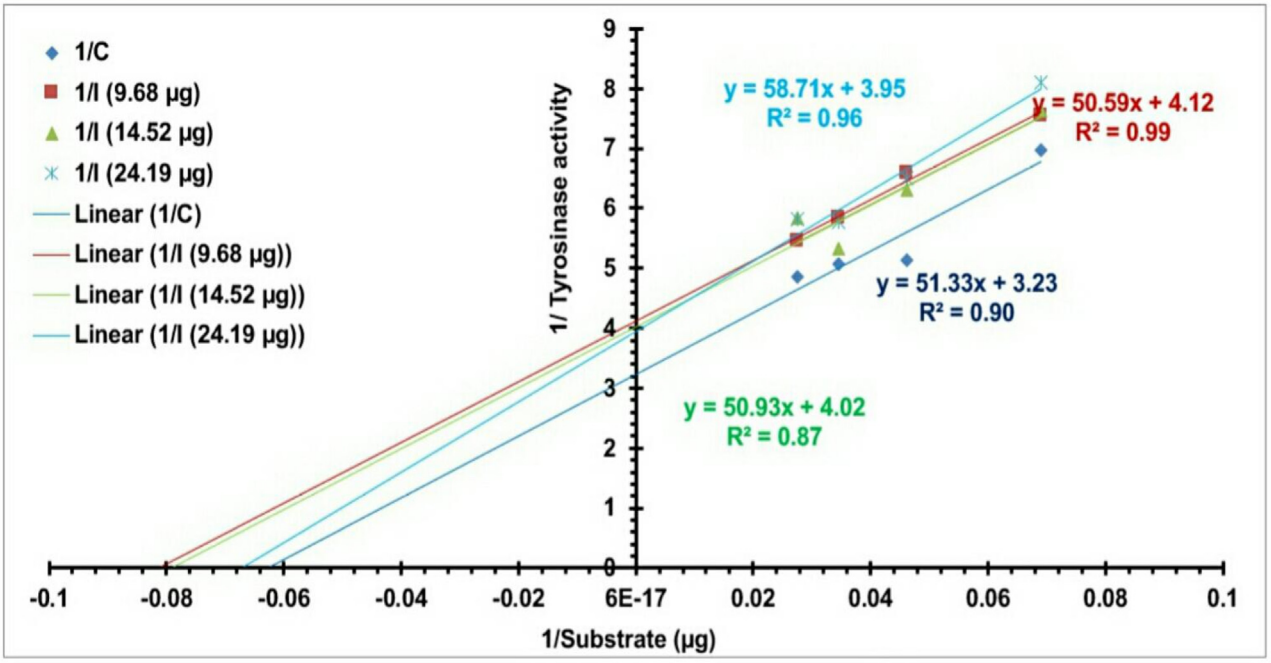
Tyrosinase inhibitor potency of protein isolates and the prepared peptides, ultrafiltration fractions F3, F2, F1 and HPLC collected peaks was determined as described by Fawole et al. (2012) with some modifications. L-tyrosine and L-DOPA were used as the substrate to determine the monophenolase and diphenolase activities of tyrosinase. Kojic was used as positive control. In a 96-well micro plate, 50 μL of sample was mixed with 30 μL of tyrosinase in phosphate buffer, pH 6.5. After 5 min incubation, 0.1 mL of 1 mM substrate was added to the reaction mixtures and incubated further for 10–30 min at 37 °C. Absorbance values of the wells were then determined at 492 nm using a micro plate reader. Tyrosinase inhibitor potency was represented as μg kojic Equivalent per μg protein or peptides. All analyses were at least performed in triplicate.

2.11. Determination of broad bean monophenolase inhibitor molecular weight

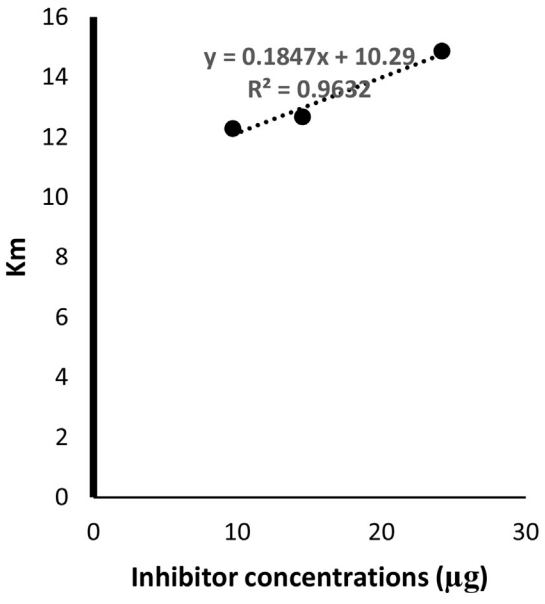
Broad bean monophenolase inhibitor was subjected to separation by High performance size exclusion chromatography (HPSEX) using the methods stated by Koza and Fountain (2013) with the following condition:, flow rate 1 ml/min; Agilent 1100 series (Waldborn, Germany), quaternary pump (G1311A), Degasser (G1322A), Thermo stated Autosampler (G1329A), Variable wave length detector (G1314A); and column: Zorbax 300SB C18 column (Agilent Technologies, USA). Detection was carried out at wave lengths 280, 214 or 195 nm depending on samples content nature. The solvent system consisted of 25 mM sodium phosphate, pH 6.8, and 30% ACN. The injection was carried out under ambient temperature. Different standards proteins [tyrosine Mwt 181.19, tripeptide (Hip-His-Leucine) Mwt 429.47, lysozyme Mwt1 4307 and carbonic anhydrase Mwt 30,000 Da] were assayed for standard molecular weight calibration curve on HPSEX.

2.12. Kinetics of broad bean monophenolase inhibitor

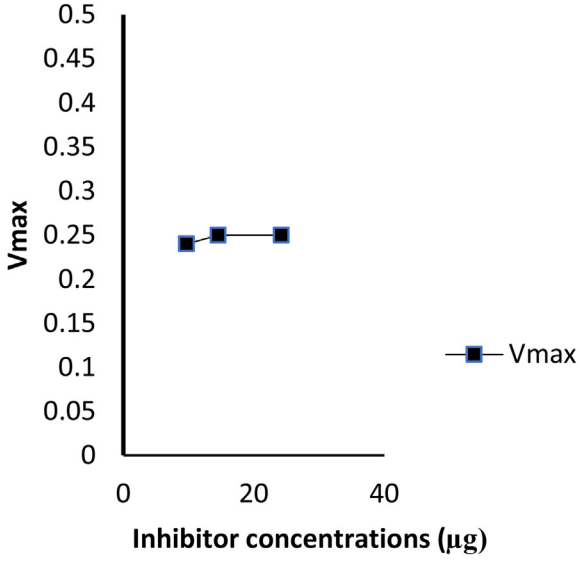
In presence of broad bean monophenolase inhibitor, apparent Michaelis-Menten constant and maximum velocity (Km, Vmax) of tyrosinase enzyme towards L-tyrosine were determined according to the



A. Lineweaver-Burk plot for the kinetic pattern of tyrosinase inhibitor.



B. The dependence of the K_m on tyrosinase inhibitor.



C. The independence of the V_{max} on tyrosinase inhibitor

Fig. 3. Kinetic pattern of broad bean tyrosinase inhibitor.

method of Kubo et al. (2003). Different concentrations of broad bean inhibitor were added to different concentrations of substrate (L-tyrosine) from 40 to 100 µg per reaction mixture. Adequate amount of tyrosinase was added and the absorbance was determined at 492 nm after 10 min.

2.13. Statistical analysis

The results were expressed as a mean ± SD, (standard deviation), n = 3 for each analysis. Data was analysed statistically using Student's t-test (2 tailed) by SPSS program.

3. Results

3.1. Peptide preparation

Fresh broad bean pods with a good percentage of storage protein were selected. They were subjected to protein extraction. The broad bean pods possessed a good amount of protein per gram of dry waste (Table 1). Immobilized lettuce protease, used to successfully hydrolyse the broad bean protein at pH 10, yielded 586 ± 38.3 μg peptides/g dry broad bean pods. The amount of peptides produced and their tyrosinase (monophenolase and diphenolase) inhibitor potency were measured *in vitro*. Tyrosinase inhibitory potency was expressed as microgram kojic equivalent (μgKE). The broad bean hydrolysate had a tyrosinase (monophenolase and diphenolase) inhibitor potency higher than that of broad bean protein by 1.6 times.

It is imperative to control and optimize the hydrolysis of broad bean protein to obtain maximum biologically active peptides. The hydrolysis rate was high during the first 60 min, and then it reached a steady-state phase in which no apparent hydrolysis took place. After 120 min of hydrolysis, the hydrolysis rate reached 80% of its original rate (data not shown). The highest peptide production was obtained at 4.5 μg broad bean protein/reaction mixture and 70 °C (Table 2). The affinity of the immobilized protease toward broad bean protein was high, with an apparent K_m value of 3.92 μg/R.M. and V_{max} value of 41.61 U/R.M. (Fig. 1).

3.2. Fractionation of the prepared broad bean hydrolysate

Enzymatic hydrolysis of broad bean protein as a substrate generated a complex mixture of active peptides. To purify bioactive peptides, broad bean hydrolysate was fractionated by ultrafiltration into three fractions according to molecular weight. The yields of F_3, F_2, and F_1 fractions were 47.1, 14.0, and 38.90%, respectively (Table 3a).

Each fraction was assayed for its tyrosinase (monophenolase and diphenolase) inhibitor potency. All fractions showed varying types of activities. The F_1 fraction had the most diphenolase inhibitor potency, while the F_2 fraction showed no diphenolase inhibitor potency. Moreover, fractions F_2 and F_1 were able to inhibit monophenolase activity with values of 135.8 and 111.4 μgKE/μg peptides, respectively.

3.3. HPLC

The peptides presented in fraction F_2, which displayed the highest monophenolase inhibition activity with absence of diphenolase inhibitory activity, were chosen for further purification by RP-HPLC. The RP-HPLC profile revealed many peaks due to the abundance of peptides generated. During the elution period, fractions (1 mL) were collected, and their monophenolase inhibition activities were tested. Only the major peak eluted at 10 min showed high monophenolase inhibition activity (Fig. 2). It was selected for further fractionation by RP-HPLC. The third RP-HPLC elution profile revealed one peak with high monophenolase inhibitor potency, 11,275 μgKE/μg peptides (Table 3b).

3.4. HPSEXC

Standard proteins (tyrosine, tripeptide [Hip-His-Leucine], lysozyme, and carbonic anhydrase) and broad bean monophenolase inhibitor were analysed using HPSEXC. There was a linear relationship between the log molecular weight of the standard proteins and their retention time, with a correlation coefficient (R^2) of 0.9995. The molecular weight of the broad bean inhibitor was calculated from the standard curve of standard proteins. It showed one peak with a retention time of 3.172 min and a calculated molecular weight of 26.102 kDa (Table 4).

3.5. Kinetics of broad bean tyrosinase inhibitory peptides

The oxidation of L-tyrosine by tyrosinase was evaluated in the presence of the prepared broad bean inhibitory peptides. The nature of inhibition was evaluated using the double reciprocal Lineweaver-Burk plot in the presence and absence of the inhibitor (Fig. 3). K_m increased with increasing monophenolase inhibitor concentrations, while V_{max} was unchanged, which indicated the broad bean inhibitor as a competitive inhibitor type.

4. Discussion

Increasing interest in the recycling of plant waste is reflected by the number of reports for its re-utilization. Plant waste is abundant, low cost, available, and continuously regenerated. Broad bean pods were chosen due to containing an appreciable amount of protein. Their protein content depends on the cultivar and growing conditions (Ahmad et al., 2012). Soluble proteins were extracted from broad bean pods using the salting out method. This method avoided the harmful effect of irreversible protein denaturation, as previously reported (Whitford, 2013).

The production of peptides was accomplished through enzymatic hydrolysis of the protein isolates by the previously prepared immobilized protease (Ali et al., 2016). For maximum production of hydrolysate (peptides), an optimized hydrolysis process, including incubation temperature and protein concentration, was established. Optimum production of peptides was observed at 70 °C of hydrolysis reaction, as similarly reported by Adebiyi et al. (2008) from rice bran. Optimization of enzymatic hydrolysis of protein for high-yield production of peptides is an important step to judge industrial application (Nilsang et al., 2005). The nature of protein and protease specificity highly influenced the molecular weight and biological properties of the peptides produced. High-yield production of peptides was important in judging industrial application. The highest peptide production was obtained at 4.5 μg broad bean protein at 70 °C and pH 10.

The amount of peptides produced and their tyrosinase (monophenolase and diphenolase) inhibitor potency were measured *in vitro*. Tyrosinase inhibitory potency was expressed as μgKE. The broad bean hydrolysate had tyrosinase (monophenolase and diphenolase) inhibitor potency higher than that of the broad bean protein by 1.6 times. Similarly, pedalitin isolated from *Rabdosia serra* and mimosine from Pacific white *Litopenaeus vannamei* had monophenolase and diphenolase inhibitors (Lin et al., 2011; Nirmal and Benjakul, 2011).

Ultrafiltration is one of the important purification steps to separate bioactive peptides based on their molecular weights. The prepared hydrolysate H was fractionated into F_3 (100 kDa), F_2 (10–100 kDa), and F_1 (below 10 kDa) fractions. Fraction F_3 was expressed as a mild hydrolysis product. Fraction F_2 could be expressed as a moderate hydrolysis product, where as fraction F_1 was expressed as an extensive hydrolysis product. Tyrosinase inhibitor from yeast peptides was purified through successive ultrafiltration with 10, 5, and 1 KDa ultrafiltration membranes (Wu et al., 2014).

The fraction with high monophenolase inhibitor potency, F_2, was further fractionated by three successive RP-HPLCs followed by HPSEXC to finally achieve a single peak, confirming its purity, with a molecular weight of 26.102 kDa. It had superior monophenolase inhibitor potency compared to that of the original protein. Several peaks were detectable by RP-HPLC in fraction F_2, confirming the hydrolysis of the broad bean protein and generation of several peptides. The molecular weight value of tyrosinase inhibitory peptides from egg white lysozyme was 14 kDa (Li et al., 2006), while that of protein was 66 kDa (Vijayan et al., 1982). Tyrosinase inhibitor from yeast peptides showed a molecular weight range of 200–800 kDa (Wu et al., 2014).

Monophenolase inhibitor exhibited a competitive type of inhibition. It could be qualified as having potent inhibitory activity against the monophenolase activity of tyrosinase and successfully reducing its

oxidation capacity. It competed against L-tyrosine to bind to the active site of the tyrosinase. Similarly, essential oils extracted from the *Citrus grandis* medicinal plant and esculetin (coumarin) isolated from *Euphorbia lathyris* seeds showed competitive tyrosinase inhibition (Aumeeruddy-Elalfi et al., 2016; Masamoto et al., 2003). Agartine and arbutin showed monophenolase inhibitor potency of a competitive type (Espin et al., 1998; Jin et al., 1999), while natural isolated tyrosinase inhibitor isoflavene "glabrene" extracted from liquorice roots showed uncompetitive inhibition (Nerya et al., 2003), and tyrosinase inhibitor from egg white lysozyme was of a mixed inhibitor type (Li et al., 2006). Uncompetitive inhibition of tyrosinase was reported for synthetic inhibitors such as [1,4'] Bipiperidinyl-1′-yl-naphthan-2-yl-methanone (I), [1,4'] Bipiperidinyl-1′-yl-4-methylphenyl-methane (II), octyl 3,4-dihydroxybenzoate, and heptyl 3,4-dihydroxybenzoate (Karbassi et al., 2004; Pan et al., 2011).

At present, tyrosinase inhibitors such as combination of ascorbic and citric acids or 4-hexylresorcinol are used as anti-browning agents in the food industry (Loizzo et al., 2012), where as Kojic acid is used as a skin-whitening component in the cosmetic industry (Chang, 2013). Safety considerations have increased interest in the use of proteins and peptides as tyrosinase inhibitors (Schurink et al., 2007). Our results suggest that broad bean pods are a good source of tyrosinase inhibitory peptides, which exhibit therapeutic potential for curing or preventing some diseases. The novelty of our work needs more study to unravel the binding and the effect of fraction F_2 on the tyrosinase enzyme.

5. Conclusion

The results of this study indicate that enzymatic proteolysis is a good method for obtaining protein hydrolysate with biological activities higher than that of the parent protein. The tyrosinase inhibition activity of broad bean protein was improved through enzymatic hydrolysis with immobilized protease. The broad bean hydrolysate exhibited different tyrosinase (monophenolase and diphenolase) inhibitor potency of varying degrees according to its molecular weight. Fraction $F_{2,}$ with high monophenolase inhibitor potency, was purified by RP-HPLC and HPSEXC. It is a potent competitive inhibitor type. It competed against L-tyrosine to bind to the active site of the tyrosinase and successfully reduce its oxidation capacity. Thus, the generated peptides may be used in the nutraceutical field, giving rise to new natural and healthier bioactive products.

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

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