รายงานวิจัยฉบับสมบูรณ์

เรื่อง

การพัฒนาสารต้นแบบสำหรับการรักษาโรคจากโปรตีนและเปปไทด์ :

กรณีศึกษาหอยหวานและขนไก่ป่น

นำเสนอโดย

รองศาสตราจารย์ ดร. อภิชาติ กาญจนทัต

สถาบันวิจัยเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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ABSTRACT (THAI)

ไทโรซิเนสเป็นเอนไซม์ที่มีคอปเปอร์เป็นองค์ประกอบ ซึ่งจะทำหน้าที่เร่งปฏิกิริยา 2 ขั้นตอน ซึ่งเกิดในช่วงเริ่มต้นของการสังเคราะห์เม็ดสีเมลานินสำหรับสัตว์เลี้ยงลูกด้วยน้ำนม งานวิจัยเกี่ยวกับสารยับยั้งไทโรซิเนสจึงกลายเป็นสิ่งที่มีความน่าสนใจเนื่องจากมีศักยภาพในการนำไปใช้ในผลิตภัณฑ์เพื่อผิวขาว ในงานวิจัยนี้ได้ศึกษาฤทธิ์ยับยั้งไทโรซิเนสของโปรตีนไฮโดรไลเสตที่เตรียมจากขนไก่ป่น โดยเตรียมจากเพพซิน-แพนครีเอตินและปาเปนในการย่อยสลาย คัดแยกโปรตีนไฮไดรไลเสตที่ได้ตามขนาดโมเลกุลด้วยเทคนิคอัลตราฟิลเตรชัน ผลการศึกษาพบว่าโปรตีนไฮไดรไลเสตที่เตรียมโดยใช้เพพซิน-แพนครีเอตินที่มีขนาดโมเลกุลน้อยกว่า 3 กิโลดาลตัน มีฤทธิ์ในการยับยั้งไทโรซิเนสดีที่สุด โดยแสดงค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งได้ร้อยละ 50 ของกิจกรรมโมโนฟีโนเลส เท่ากับ 5.780 ± 0.188 ไมโครกรัมต่อมิลลิลิตร และกิจกรรมไดฟีโนเลส เท่ากับ IC_{50} 0.040 ± 0.024 ไมโครกรัมต่อมิลลิลิตร ตามลาดับผลการศึกษาจลนพลศาสตร์ของกิจกรรมยับยั้งไทโรซิเนสพบว่ามีการยับยั้งแบบไม่แข่งขัน มีค่าสัมประสิทธิ์การยับยั้งเท่ากับ 18.149 และ 27.189 ไมโครกรัมต่อมิลลิลิตร สำหรับกิจกรรมของโมโนฟีโนเลสและไดฟีโนเลส ตามลำดับ นอกจากนี้ผลจากการศึกษาในเซลล์เมลาโนไซต์พบว่าโปรตีนไฮไดรไลเสตที่เตรียมโดยใช้เพพซิน-แพนครีเอติน ที่มีขนาดโมเลกุลน้อยกว่า 3 กิโลดาลตันยังแสดงการยับยั้งดีที่สุดต่อการอยู่รอดของเซลล์ที่สุด โดยแสดงค่าความเข้มข้นต่าสุดที่สามารถยับยั้งได้ร้อยละ 50 เท่ากับ 1.124 ± 0.288 ไมโครกรัมต่อมิลลิลิตร สามารถยับยั้งกิจกรรมของไทโรซิเนสและยับยั้งการสร้างเมลานินได้เท่ากับ 50.493 เปอร์เซ็นต์ และ 14. 680เปอร์เซ็นต์ ตามลำดับ นอกจากนี้โปรตีนไฮโดรไลเสตยังได้ถูกศึกษาผลต่อการตายแบบอะพอพโตซิสในเซลล์ และตรวจเอกลักษณ์ของโปรตีนไฮโดรไลเสตที่มีขนาดโมเลกุลน้อยกว่า 3 กิโลดาลตัน ด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง และเทคนิคแมสสเปกโตรเมตรี ตามลำดับ การวิจัยเพื่อค้นหาฤทธิ์ยับยั้งไทโรซิเนสจากโปรตีนไฮไดรไลเสตที่เตรียมจากขนไก่ป่นนี้ มีความเป็นไปได้ที่จะนำไปประยุกต์ใช้เป็นส่วนประกอบสำหรับผลิตภัณฑ์เพื่อผิวขาวต่อไป

คำสำคัญ: ขนไก่ป่น; การยับยั้งไทโรซิเนส; โปรตีนไฮโดรไลเสต; การยับยั้งการเกิดเม็ดสีเมลานิน

ABSTRACT (ENGLISH)

Tyrosinase is a copper-containing enzyme that controls mammalian melanogenesis. Tyrosinase inhibitors are important for their potential application in cosmetic products. Chicken feather meal is a rich source of amino acids, which have been linked with tyrosinase inhibition activity. This study investigated the tyrosinase inhibitory properties of protein hydrolysates prepared from chicken feather meal. Protein hydrolysates prepared by pepsin-pancreatin with MW < 3 kDa exhibited strong tyrosinase inhibition activity for both monophenolase (IC_{50} 5.780 ± 0.188 μg/mL) and diphenolase activities (IC_{50} 0.040 ± 0.024 μg/mL) in a cell-free mushroom tyrosinase system. These samples were uncompetitive inhibitors with Ki values of 18.149 and 27.189 μg/mL in monophenolase and diphenolase activities, respectively. A cell culture model showed that this hydrolysate had the strongest inhibition on the viability of B16F10 cells (IC_{50} 1.124 ± 0.288 μg/mL) and 0.210 μg/mL of the sample exhibited inhibition of tyrosinase activity by 50.493% and melanin synthesis by 14.680% compared to the control.

Keywords: chicken feather meal; tyrosinase inhibition; protein hydrolysates; melanogenesis

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1. INTRODUCTION

Melanin is a unique and ubiquitous pigment widely known for its ability to help protect the human skin from the damaging effects of ultraviolet (UV) radiation through the absorption of UV sunlight and removal of reactive oxygen species. The pigment is secreted by melanocyte cells, which are found within the basal layer of the dermis (1). Melanin is synthesized through melanogenesis by melanocytes within the skin and hair follicles. Melanosomes are specialized organelles similar to lysosomes found within melanocytes, and contain several enzymes that mediate the production of two basic types of melanin: eumelanin (brown or black) and pheomelanin (red or yellow). Indeed, the human skin color is determined by the type, amount, and distribution of melanin in the surrounding keratinocytes (2). However, if exceptionally high amounts of melanin accumulate in specific areas of the skin, the result is pigmented patches – such as freckles, ephelide, senile lentigines, and melasma – which can pose an aesthetic problem. This can be further compounded by the social and cultural implications that exist in several Asian countries pertaining to pale skin, with a light complexion being widely considered as ideal. Building on this, many skin whitening products have been successfully launched in the market, and the skin whitening and lightening industry is experiencing continual growth (3).

A series of oxidative reactions that involve tyrosine in the presence of the enzyme tyrosinase produce melanin. A copper-containing polyphenol oxidase, tyrosinase (EC 1.14.18.1), is found throughout nature, but only in melanocytes with a molecular weight of 60–70 kDa among mammals (2). Tyrosinase is capable of accepting a broad range of p-substituted monophenolic and diphenolic substrates, of which L-tyrosine and 3, 4-dihydroxy-L-phenylalanine (L-DOPA) are the natural precursors of melanin. As the major enzyme in melanogenesis, tyrosinase catalyzes two reactions in melanin synthesis –the oxidation of DOPA to dopaquinone (diphenolase activity) and the hydroxylation of tyrosine to DOPA (monophenolase activity) (3, 4). In addition to being highly reactive,

quinones are able to spontaneously polymerize and form high-molecular-weight compounds or the brown pigments of eumelanin and pheomelanin. Tyrosinase-related proteins TRP-1 and DCT/TRP-2 catalyze the melanin biosynthesis steps controlling the type of melanin produced (4).

Apart from hair color and skin pigmentation, tyrosinase is also responsible for skin anomalies in mammals, with examples including both hypo (vitiligo)- and hyper (flecks or freckles)-pigmentation. More recently, greater attention has been directed toward tyrosinase inhibitors as components of medicinal and cosmetic goods used in the prevention or treatment of pigmentation disorders (1). Widely considered to be safe and without adverse side effects, tyrosinase inhibitors from natural sources have consequently come to the fore. Indeed, proteins and peptides from milk, wheat, honey, and silk have been investigated and shown to successfully inhibit tyrosinase activity (5). The conclusion to be drawn from such studies as a whole is that natural sources of peptides and protein hydrolysates are potential tyrosinase inhibitors.

Protein hydrolysate is produced by the digestion of proteins, which can be broken down by enzymatic or chemical hydrolysis into peptides of different sizes and free amino acids. The technological advantages of protein hydrolysates include improved solubility, heat stability, and a relatively high resistance to precipitation by many agents such as pH or metal ions. Additionally, the pharmaceutical, human nutrition, and cosmetic industries have all utilized protein hydrolysates with considerable success (6-8).

Chicken feathers are a protein-rich waste product of the poultry processing industry (9-12). Raw feathers contain a high proportion of keratin protein, which contains cysteine disulfide bonds and can therefore be used as a good starting material for protein hydrolysates, which can exhibit inhibitory activity toward tyrosinase. We speculate whether chicken feather meal can serve as a new source of protein hydrolysate, which may have tyrosinase inhibition activity. Consequently, this study aims to study the potential tyrosinase inhibitory activity of protein hydrolysate from chicken feather meal. The ability of protein hydrolysates to inhibit tyrosinase in melanin biosynthesis is investigated using a cell-free mushroom tyrosinase system and a cell culture model.

2. EXPERIMENTALS

Biological and chemical materials Chicken feather meal from Betagro Science Center Co., Ltd. (Pathumthani, Thailand), was ground to a small size and dried at 60°C overnight. The feather meal was then filtered through a 150-micron sieve. Bovine serum albumin (BSA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, phenylmethanesulfonylfluoride (PMSF) L-DOPA, L-tyrosine, and tyrosinase from mushroom were purchased from Sigma-Aldrich (Missouri, USA). All other chemicals used in the investigation were of analytical grade.

Amino acid content analysis The amino acid content of the chicken feather meal was determined based on the standard AOAC 994.12 acid hydrolysis method. The samples were mixed with 5 mL of 6 N HCl and placed in a heating block at 110°C for 24 h to liberate the individual amino acids before reversed-phase high-performance liquid chromatography (RP-HPLC) analysis. The analyses were carried out on a C_{18} column (4.6 mm × 250 mm, 5 μm) (Waters, Ireland), using a gradient of Buffer A (12.5 mM phosphate buffer, pH 7.2) and Buffer B (Buffer A containing 50% acetonitrile) with a flow rate of 1 mL/min.

Protein hydrolysate preparation Chicken feather meal was mixed with 20 mM potassium phosphate buffer at pH 7.2 at 0.0125 g/mL. The suspension was stirred overnight at 4°C. The protein hydrolysate was then prepared following the method of Torres-Fuentes et al. (13).

Pepsin-pancreatin Crude protein was adjusted to pH 2.5 with the addition of 1 M HCl and then mixed with pepsin using an enzyme:substrate ratio of 1:20 (w/v). The hydrolysis was carried out for 180 min at 37°C with shaking (180 rpm), and then inactivated by adding 1 M NaOH to pH 7.5. Pancreatin was then added to a 20:1 (g/g) substrate:enzyme

ratio and shaken (180 rpm) for 180 min at 37°C. Hydrolysis was stopped by heating at 80°C for 20 min. Hydrolysates were clarified by centrifugation at 15,000 × g for 30 min at 4°C and kept at –20°C until use.

Papain Crude protein was adjusted to pH 6.5 and mixed with papain using an enzyme:substrate ratio of 1:20 (w/v). The hydrolysis was carried out for 240 min at 65°C with shaking (180 rpm) and stopped by heating at 80°C for 20 min. Hydrolysates were clarified by centrifugation at 15,000 × g for 30 min at 4°C and kept at –20 °C until use.

Molecular weight cut-off by ultrafiltration membrane The protein hydrolysates were fractionated by ultrafiltration membranes using a bioreactor system (Amersham Biosciences, Sweden). The suspension of protein hydrolysate prepared from chicken feather meal was pumped through a range of nominal-molecular-weight cut-off membranes of 10, 5, and 3 kDa Pellicon XL filter unit (Millipore) in order of decreasing pore size. Five fractions were collected from the membrane filtration: retentate from 10 kDa membrane (MW > 10 kDa), retentate from 5 kDa membrane (MW 5–10 kDa), retentate from 3 kDa membrane (MW 3–5 kDa), permeate from 5 kDa membrane (MW < 5 kDa), and permeate from 3 kDa membrane (MW < 3 kDa).

Tyrosinase inhibition assay The tyrosinase inhibition assay was modified from the method used by Batubara et al. (14). The protein hydrolysate (35 μL) was mixed with 15 μL of tyrosinase (333 U/mL in phosphate buffer (50 mM, pH 6.5)) and incubated at room temperature for 5 min. Then, 55 μl of substrate (12 mM L-DOPA) was added to each tube and incubated for 30 min. The absorbance was determined at 510 nm using a microplate reader. Kojic acid was used as the positive control. The percentage inhibition of tyrosinase activity was calculated using the following equation:

Inhibitory effect on tyrosinase (%) =

$$\frac{[(\text{Abs control} - \text{Abs blank}) - (\text{Abs sample} - \text{Abs background})]}{(\text{Abs control} - \text{Abs blank}),} \times 100$$

where Abs control is the absorbance of control (no sample), Abs sample is the absorbance of the sample, Abs background is the absorbance of the background (color of sample), and Abs blank is the absorbance of blank (deionized water). IC_{50} is the concentration of protein hydrolysate in which 50% of enzyme activity is inhibited.

Cell culture The mouse melanocyte cell line B16F10 was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1.5 g/l $NaHCO_3$, 2 mM L-glutamine, 10 μg/mL penicillin, 10 μg/mL streptomycin, and 0.25 μg/mL amphotericin B, and then incubated at 37°C with 5% CO_2 in a humidified atmosphere. Cells were sub-cultured at a ratio of 1:3 on every third or fourth day.

Determination of protein hydrolysate toxicity in melanocytes (MTT assay) B16F10 cells in complete DMEM were added into the wells of a 96-well plate (5×10^3 cells/well in 200 μL) and incubated overnight in a 37°C incubator with 5% CO_2. A test sample was then added to each well and incubated for another 3 days. The treated cells were then labeled with a 5 mg/mL MTT dye reagent (10 μL/well) and incubated at 37°C for 4 h. The formazan precipitates were dissolved in DMSO (150 μL/well), and after addition of 25 μl of 0.1 M glycine, the absorbance values of the supernatant were measured at 540 nm.

Determination of melanin content in melanocytes Melanin content determination was performed based on Si et al.'s (15) method, with some modifications. B16F10 cells in complete DMEM were added to the cell culture flasks (1×10^5 cell/flask in 5 mL) and incubated overnight at 37°C with 5% CO_2. A test sample and the positive-control kojic acid were then added to each flask and incubated for another 3 days. The treated cells were then harvested and washed twice with phosphate-buffered saline ((PBS) pH 7.4). Finally, all cells were lysed with 500 μL of 1 N NaOH. After 1 h of incubation at 90°C, the lysates were centrifuged at 3,000 g for 10 min and the absorbance values were measured at 405 nm.

Cellular tyrosinase inhibition assay Cellular tyrosinase inhibition assay measurement was modified using Si et al.'s (15) method. B16F10 cells in complete DMEM were added to the cell culture flasks (1×10^5 cell/flask in 5 mL) and incubated overnight at 37°C with 5% CO_2. The cells were then treated with a test sample and positive-control kojic acid. After 3 days of incubation, the treated cells were harvested and washed twice with cold PBS (pH 7.4). Finally, all cells were lysed with PBS (pH 6.8) containing 1% (w/v) Triton X-100 and 1 mM PMSF. The enzymatic assay was commenced by mixing 100 µL of cell extract with 100 µl of L-DOPA (2 mM) in a 96-well plate. After 1 h of incubation at 37°C, the absorbance values were measured at 490 nm.

Isolation of peptides with tyrosinase inhibitory activity Protein hydrolysates with MW < 3 kDa were analyzed by HPLC. The analyses were conducted on a Luna C18 (4.6 mm × 250 mm) column. The mobile phases consisted of solvent A (0.1 (w/v) trifluoroacetonitrile acid) and solvent B (70 % (v/v) acetonitrile in water containing 0.05% (w/v) trifluoroacetonitrile acid). The flow rate was set at 0.7 mL/min and the detection system monitored absorbance at 280 nm. The sample injection volume was 50 µL.

Identification of tyrosinase inhibitor peptides The liquid chromatography coupled with the tandem mass spectrometry (LC/MS/MS) method was used to identify the amino acid sequence of each internal fragment of protein hydrolysate. The LC/MS/MS system consists of an LC part (Dionex Ultimate 3000, Thermo Scientific, USA) in combination with an electrospray ionization/quadrupole ion trap mass spectrometer (Model Amazon SL, Bruker, Germany). The LC separation was performed on a reversed-phase column (Hypersil GOLD 50 mm × 0.5 mm, 5 µm C18), protected by a guard column (Hypersil GOLD 30 mm × 0.5 mm, 5 µm C18). Mobile phase A consisted of water/formic acid (99.9:0.1, v/v) and B acetonitrile (100, v). Analyte separation was performed under gradient conditions of 5–80% B over 50 min at a flow rate of 100 µl/min. Mass spectral

data from 300 to 1500 *m/z* were collected in the positive ionization mode. All data were processed and submitted to a MASCOT (http://www.matrixscience.com) search of the NCBI database (http://blast.ncbi.nlm.nih.gov).

Kinetic analysis of tyrosinase inhibition The assay was performed using the same protocol as the measurement for tyrosinase inhibition activity, apart from changes in the concentrations of the substrate. The reaction mixture consisted of different concentrations of the substrate (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 1.8, and 2.0 mM for L-tyrosine and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 mM for L-DOPA) and mushroom tyrosinase (333 U/mL in 50 mM phosphate buffer, pH 6.5). Three different concentrations of the inhibitors were added to each reaction mixture and incubated at 37°C. The Lineweaver-Burk plot method was used to determine the reaction kinetics (16).

Protein content determination The protein content was determined by Bradford's procedure (17). BSA was used as the standard with nine concentrations (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 μg/mL) to generate the standard curve.

Statistical analysis The results of all measurements were expressed as the mean ± standard deviation. All investigations were performed in triplicate. IC_{50} values were calculated using GraphPad Prism (Version 6.00, GraphPad Software Inc., La Jolla, CA, USA) for Windows. Statistical analysis for comparing the results was performed by Student's t-test or one-way ANOVA, followed by Tukey's test. $P < 0.05$ was considered to represent statistical significance. All statistical analyses were performed using the statistics program SPSS version 22.

3. RESULT AND DISCUSSION

Amino acid content of chicken feather meal The amino acids detected included alanine, arginine, glycine, aspartic acid, valine, cystine, glutamic acid, leucine,

isoleucine, histidine, threonine, proline, lysine, methionine, serine, phenylalanine, tyrosine, and tryptophan in different amounts. A previous report by Schurink et al. (5) demonstrated that effective tyrosinase inhibitory peptides contain arginine, phenylalanine, valine, alanine, and leucine and these amino acids are considered important in the inhibition of tyrosinase. Apart from the hydrophobic residues, peptides containing the polar, uncharged residues cysteine and serine also showed significant inhibitory activity. Ishikawa et al. (18) noted that some amino acids, such as L-alanine, glycine, L-isoleucine, and L-leucine, possess beneficial effects in the disruption of melanogenesis. The chicken feather meal powder also only contained small amounts of tyrosine. Tyrosine is a substrate of tyrosinase in melanogenesis, and therefore, should not be present in a large amount in a tyrosinase inhibitor because it might increase the concentration of the substrate and reduce inhibitory activity. The chicken feather meal powder also contained a large quantity of serine. Note that both sericin (silk protein) and phosvitin (phosphoglycoprotein present in egg yolk) show high levels of serine (30–33% and 50% serine, respectively) and have demonstrated the ability to inhibit tyrosinase activity (19, 20).

Optimization of enzymatic hydrolysis conditions of chicken feather meal by pepsin-pancreatin and papain Optimization of the enzymatic hydrolysis conditions of feather meal was performed using seven substrate concentrations (0.00625, 0.0125, 0.0250, 0.0500, 0.1000, 0.2500, and 0.5000 mg/mL). The protein hydrolysate was prepared following the procedure described in the experimental section using two types of enzymes (pepsin-pancreatin and papain) and evaluated for tyrosinase inhibitory activity. The protein hydrolysate from 0.0125 g/mL substrate provided the best IC_{50} values: 5.369 ± 2.361 μg/mL of protein hydrolysate from pepsin-pancreatin hydrolysis and 17.220 ± 7.618 μg/mL from papain hydrolysis. The protein hydrolysate under this condition was selected for further study.

Tyrosinase inhibition activity of protein hydrolysate fraction The protein hydrolysate under the optimal condition was fractionated into different sizes of peptide - MW x > 10 kDa, 5–10 kDa, x < 5 kDa, 3–5 kDa, and x < 3 kDa - using ultrafiltration membranes and a Microsep Advance Centrifugal Device. All fractions were assayed for *in vitro* tyrosinase inhibitory activities to test for their ability as tyrosinase inhibitors in both monophenolase and diphenolase activities, according to the procedure described in the experimental section. The results are shown in Table 1. The protein hydrolysate prepared by pepsin-pancreatin with MW < 3 kDa showed the lowest IC_{50} value in both monophenolase (5.780 ± 0.188 μg/mL) and diphenolase activities (0.040 ± 0.024 μg/mL). In monophenolase activities, protein hydrolysates with MW < 3 kDa showed stronger tyrosinase inhibitory activity than the positive-control kojic acid (6.076 ± 0.001 μg/mL). The IC_{50} value of protein hydrolysates with MW < 3 kDa (0.040 ± 0.024) in diphenolase activities was less potent than that of kojic acid (0.034 ± 0.000 μg/mL), but the difference was not statistically significant. These results indicate that the protein hydrolysates prepared by pepsin-pancreatin with MW < 3 kDa are good tyrosinase inhibitors as they can inhibit tyrosinase using the lowest-concentration sample. Therefore, protein hydrolysates prepared by pepsin-pancreatin were selected for further investigation.

Our results were comparable with previously published findings. Wu et al. (21) found that sericin hydrolysate exhibited a tyrosinase inhibitory effect in a dose-dependent manner with an IC_{50} value of 10 mg/mL. Manosroi et al. (22) reported that silk protein (sericin) from native Thai silkworms showed tyrosinase inhibition activity with an IC_{50} value of 1.2–18.76 mg/mL. Zhuang et al. (23) noted that the tyrosinase inhibition activity of hydrolysates of jellyfish umbrella collagen (HF-2) was higher than 50% at 5 mg/mL, which showed lower tyrosinase inhibition activity than the protein hydrolysates in this study. Their studies indicated that HF-2 with MW 1000 Da < HF-2 < 3000 Da exhibited the best tyrosinase inhibitory activity, which have a size similar to that of the protein hydrolysates tested in this report.

Table 1 Fractions of protein hydrolysates prepared from chicken feather meal using pepsin-pancreatin and papain hydrolysis and their tyrosinase inhibition activity

Molecular weight (kDa)	Tyrosinase inhibition IC_{50} (μg/mL)*			
	Monophenolase activity		Diphenolase activity	
	Pepsin-pancreatin	papain	Pepsin-pancreatin	papain
crude protein	22.697 ± 4.177[b]	22.697 ± 4.177[d]	21.363 ± 5.940[b,c]	21.363 ± 5.940[c]
crude hydrolysate	34.627 ± 1.845[d]	8.617 ± 0.203[a]	5.369 ± 2.361[a]	17.220 ± 7.618[b,c]
> 10 kDa	63.277 ± 6.046[e]	8.546 ± 0.167[a]	26.753 ± 19.388[c]	35.583 ± 9.694[d]
5-10 kDa	28.713 ± 1.320[c]	15.873 ± 0.160[b]	10.561 ± 3.689[a,b]	40.053 ± 5.871[d]
< 5 kDa	19.857 ± 0.075[b]	18.927 ± 0.370[c]	3.927 ± 1.713[a]	5.908 ± 4.812[a,b]
3-5 kDa	19.637 ± 0.635[b]	27.930 ± 1.400[e]	1.360 ± 1.370[a]	0.997 ± 0.278[a]
< 3 kDa	5.780 ± 0.188[a]	31.190 ± 1.771[f]	0.040 ± 0.024[a]	10.776 ± 13.412[a,b,c]
Kojic acid	6.076 ± 0.001[a]	6.076 ± 0.001[a]	0.034 ± 0.000[a]	0.034 ± 0.000[a]

* All data are shown as the mean ± standard deviation, obtained from three repeated determinations

Inhibitory effect on B16F10 cells of protein hydrolysate fraction

Effect of protein hydrolysates on cell viability Our results suggest that protein hydrolysates prepared from chicken feather meal are useful tyrosinase inhibitors due to their potent enzymatic inhibitory activity. However, the effect of these hydrolysates on cells is also a highly important factor. All fractions of protein hydrolysates prepared from chicken feather meal were determined for their cytotoxicity using melanoma B16F10 cells as a model system. The B16F10 murine melanoma cell line was used in this study as it is considered a good model for studying human melanoma, which has a short population-doubling time. B16F10 cells are easy to culture and have better survival rates than human melanocyte cells (24, 25). Moreover, research using B16 melanoma cells has suggested that the mechanism of melanogenesis is similar to that in normal human melanocytes (26).

B16F10 cells were treated with six concentrations of samples in seven fractions and tested by MTT cell viability assay. The IC_{50} values of all fractions are shown in Table 2. The results showed that all fractions of protein hydrolysates have some cytotoxic activity and the cytotoxic effect was related to the size (molecular weight) of the protein hydrolysates. Protein hydrolysate with MW < 3 kDa showed the lowest IC_{50} value (1.124 ± 0.288 μg/mL).

The results of the MTT cell viability assay revealed that most cells were still viable upon treatment with sample concentrations of 0.638 and 0.213 μg/mL (Fig. 1). Samples at these concentrations were further used to test for cellular tyrosinase inhibitory activities, as a desirable skin-whitening agent should inhibit melanin synthesis in melanosomes by acting specifically to reduce the synthesis or activity of tyrosinase with little or no cytotoxicity (27). Protein hydrolysate with MW < 3 kDa at higher concentration levels (1.063, 1.488, 1.913, and 2.125 μg/mL) was not further studied due to high cytotoxicity in melanoma cells.

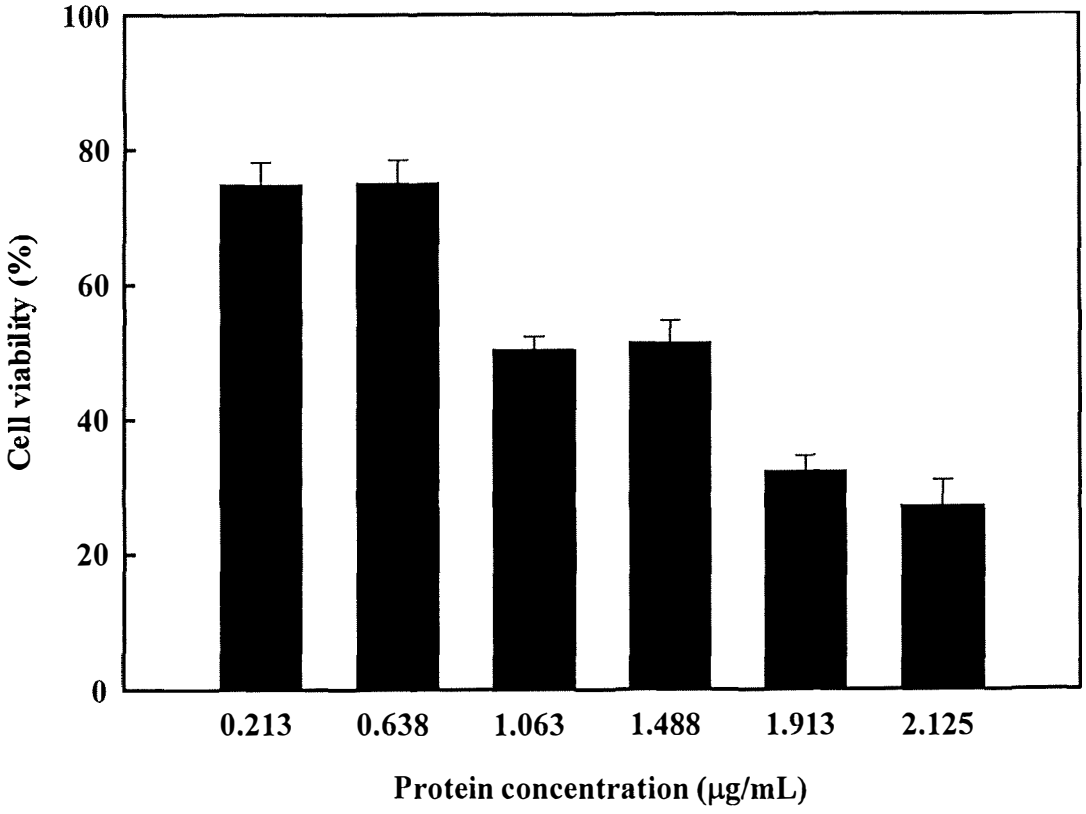

Fig. 1 Viability of B16F10 cells after treatment with various concentrations of protein hydrolysates prepared from chicken feather meal (MW < 3 kDa) for 72 h.

Table 2 Fractions of protein hydrolysates prepared from chicken feather meal using pepsin-pancreatin hydrolysis and their inhibitory effect on B16F10 cells

Molecular weight (kDa)	Melanocyte cells viability IC_{50} (μg/mL)*
crude protein	26.083 ± 0.876^{d}
crude hydrolysate	17.370 ± 2.258^{c}
> 10 kDa	24.900 ± 3.330^{d}
5 - 10 kDa	14.640 ± 1.906^{c}
< 5 kDa	$3.583 \pm 0.413^{a,b}$
3 - 5 kDa	4.786 ± 0.841^{b}
< 3 kDa	1.124 ± 0.288^{a}

* All data are shown as the mean ± standard deviation, obtained from three repeated determinations

Effect of protein hydrolysates on cellular tyrosinase activity Our results indicated that among all sample fractions, protein hydrolysates with MW < 3 kDa showed the best mushroom tyrosinase inhibition activity. Therefore, this fraction was selected for further investigation of its ability to inhibit tyrosinase activity in B16F10 cells. Samples with concentrations that showed low toxicity in cells were selected to test for cellular tyrosinase inhibition assay. The protein hydrolysate MW < 3 kDa at a concentration of 0.638 μg/mL was prepared into three dilutions and tested by cellular tyrosinase inhibition assay according to the procedure described in the experimental section. Fig. 2 demonstrates that the samples inhibited cellular tyrosinase in B16F10 cells in a dose-dependent manner: protein hydrolysates at 0.050, 0.100, and 0.210 μg/mL induced inhibition of cellular tyrosinase by 13.260, 45.295, and 50.493%, respectively. The inhibitions at 0.100 and 0.210 μg/mL significantly differed with the control, but no significant difference was observed between these two concentrations. The samples showed higher tyrosinase inhibitory activity than the kojic acid used as the positive control (data not shown).

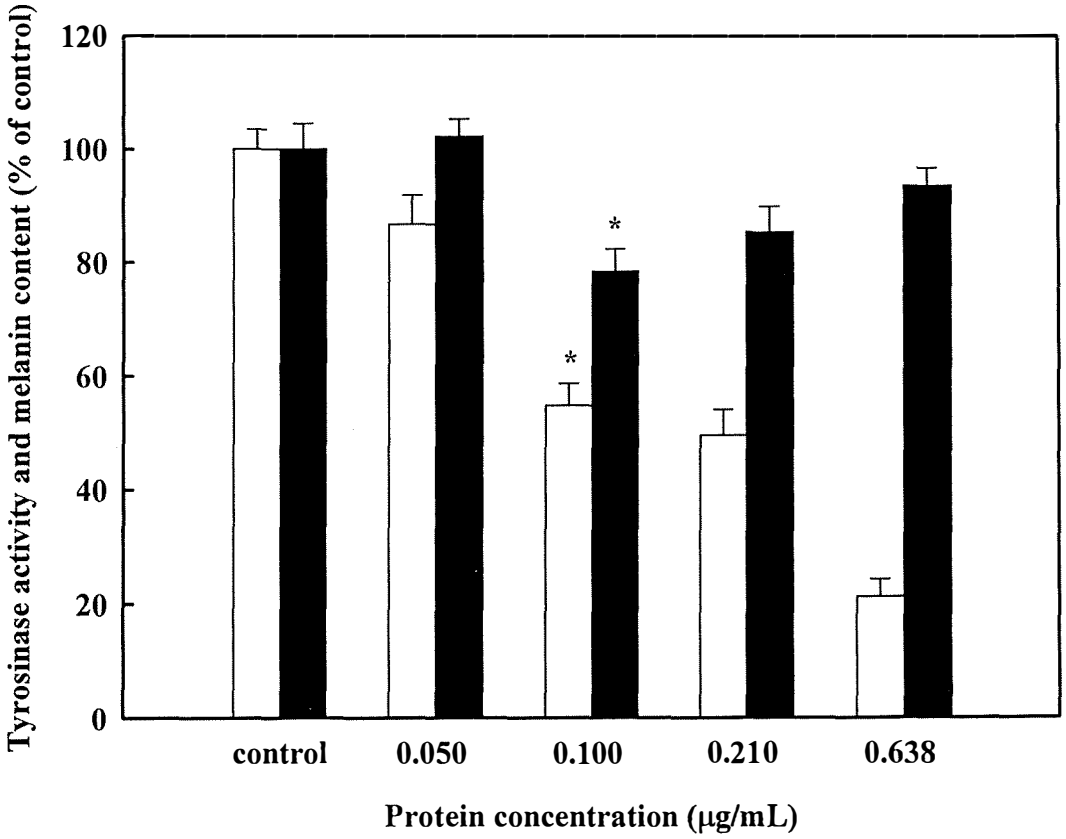

Fig. 2 Effect of protein hydrolysates on tyrosinase activity (white) and melanin content (black) in B16F10 cells. *$p < .05$ indicated a significant difference when compared to the control.

Effect of protein hydrolysates on melanin synthesis in B16F10 cells The protein hydrolysate prepared from chicken feather meal with MW < 3 kDa was investigated for its ability to inhibit melanogenesis in B16F10 cells. Melanoma cells were treated with three concentrations of samples (0.210, 0.100, and 0.050 μg/mL) and melanin content was measured according to the procedure described in the experimental section. Melanin synthesis was not reduced in B16F10 cells treated with a sample concentration of 0.050 μg/mL, while the concentrations of 0.100 and 0.210 μg/mL showed inhibition of melanin synthesis by 21.601 and 14.680%, respectively (Fig. 2). However, these results did not show statistical significance compared to the control or among concentrations. The ability of the samples to inhibit melanogenesis in B16F10 cells was lower than that of kojic acid (data not shown).

These findings show that the effect of protein hydrolysates on melanin reduction did not correlate with tyrosinase inhibition. These results were inconsistent with previous findings. When melanogenesis is affected, such as during tyrosinase inhibition, both these parameters (tyrosinase inhibition and melanin content) are coordinately increased or decreased (29). The different observation in the current study may be because melanogenesis in B16F10 cells occurs via multiple steps. Thus, the reduction in melanin formation by protein hydrolysates may not be due to direct tyrosinase inhibition. Jung et al. (20) reported that phosvitin (phosphoglycoprotein present in egg yolk) at a concentration of 50 μg/mL inhibited the tyrosinase activity of B16F10 melanoma cells by 42% and inhibited melanin synthesis by 17%; this reported tyrosinase inhibition activity was lower than that reported in the current study.

Mechanism of inhibition The inhibition modes of the tyrosinase inhibitor from protein hydrolysates of chicken feather meal prepared by pepsin-pancreatin with MW < 3 kDa in monophenolase (substrate: L-tyrosine) and diphenolase (substrate: L-DOPA) activities were investigated using Lineweaver–Burk plot analysis. Lineweaver–Burk plots show that 1/v versus 1/[S] provides a family of parallel straight lines (data not shown), indicating that inhibitors in different concentrations can change the apparent value of V_m

but the ratio of K_m/V_m remains unchanged. The results showed that both monophenolase and diphenolase activities of protein hydrolysates from chicken feather meal functioned as uncompetitive inhibitors. An uncompetitive inhibitor is an inhibitor that can bind only to the enzyme-substrate complex and not to the free enzyme (30). The inhibition constants (K_i value) were 18.149 and 27.189 μg protein/mL, respectively.

The previously studied tyrosinase inhibitors showed various inhibitory mechanisms. Chai et al. (31) reported that furoic acid could inhibit tyrosinase activities through uncompetitive inhibition. Both 2-chlorocinnamic acid and 2,4-dichlorocinnamic acid displayed a reversible and uncompetitive mechanism for tyrosinase inhibition (32). Other types of tyrosinase inhibitors, such as *Betula pendula* leaf ethanolic extract, exhibited noncompetitive inhibition on tyrosinase activities (33), and 3′,5′-di-C-b-glucopyranosylphloretin demonstrated good tyrosinase inhibitory activity by a competitive mode (34). Among the previously published tyrosinase inhibitors, while inhibitors from natural sources have generated much interest, tyrosinase inhibitors from proteins and peptides are rare. Our kinetic analysis of tyrosinase inhibitors of protein hydrolysates of chicken feather meal indicates a putative uncompetitive mechanism.

Purification of tyrosinase inhibitor peptides by RP-HPLC The protein hydrolysate of chicken feather meal prepared by pepsin-pancreatin with MW < 3 kDa showed the best result for tyrosinase inhibition. Thus, protein hydrolysates with MW < 3 kDa were subjected to peptide separation by RP-HPLC on a Shimpack C18 column using a trifluoroacetic acid/acetonitrile solvent system and detected at UV 280 nm (Fig. 3). Fractions of protein hydrolysates were collected at retention times of 0–10 min (fraction A), 10–20 min (fraction B), 20–30 min (fraction C), and 30–40 min (fraction D). All fractions were assayed for *in vitro* tyrosinase inhibitory activity to screen the maximal inhibition in diphenolase activities according to the procedure described in the experimental section. The results showed that protein hydrolysate fraction A exhibited the highest maximal inhibition at 49.655%, followed by fraction C, fraction B, and

fraction D at 31.766, 22.447, and 7.589%, respectively. We next analyzed tyrosinase inhibitor peptides in all fractions.

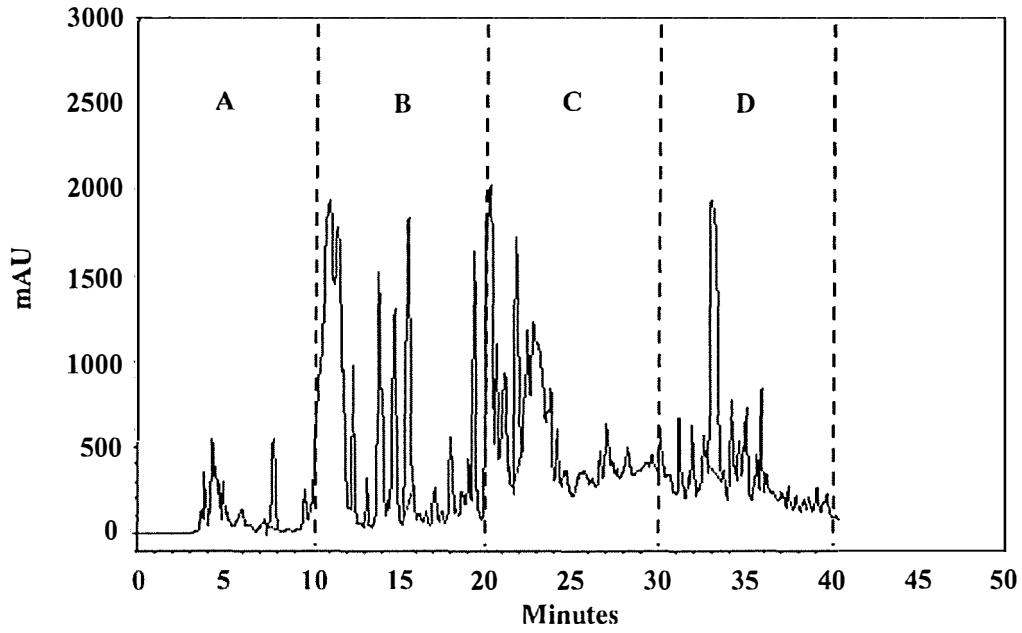

Fig. 3 RP-HPLC chromatogram of a fraction with MW < 3 kDa of protein hydrolysates from chicken feather meal, prepared by pepsin-pancreatin digestion on a Luna C18 (4.6 mm × 250 mm) column. A was collected for 0–10 min, B for 10–20 min, C for 20–30 min, and D for 30–40 min.

Identification of tyrosinase inhibitor peptides The relationship between tyrosinase inhibition activity and amino acid constituents has been previously reported (5, 18). The presence of arginine, phenylalanine, valine, alanine, leucine, glycine, serine, cysteine, and isoleucine in peptides could enhance tyrosinase inhibitory activity. Schurink et al. (5) suggested that the peptide should contain one or more arginine residues for strong tyrosinase inhibitory and binding activity. Their studies also indicated that tyrosinase inhibition is optimal when arginine and/or phenylalanine is/are combined with hydrophobic aliphatic residues such as valine, alanine, or leucine. Ubeid et al. (35) reported that oligopeptides P3 and P4 (RADSRADC and YRSRKYSSWY) showed

inhibition of mushroom and human tyrosinase activities. Oligopeptide P3 contains the combination Arg-Ala at positions 1/2 and 5/6, as well as the amino acids serine and cysteine at positions 4 and 8, respectively. In oligopeptide P4, the presence of arginine at positions 2 and 4 and serine at 3, 7, and 8 may explain the strong inhibitory activity observed for this oligopeptide.

We identified 18 peptide sequences from four fractions of protein hydrolysate with MW < 3 kDa by LC/MS/MS analysis. The results are shown in Table 3. Fractions 1, 2, 3, and 4 showed 5, 5, 5, and 3 peptide sequences, respectively. Fraction 1 had the combination Arg-Val at position 15/16 of peptide GCGYKPCDPQVIRDRVA and Arg-Leu at position 4/5 of peptide CSARLVNYGYTFGSG. The combination Phe-Ala was present at position 8/9 of peptide GCYIEGFFATLGGEIALW from fraction 2 and Arg-Val was present at position 2/3 of peptide NRVYVHPF from fraction 4. Moreover, all sequences of identified peptides showed at least one arginine, phenylalanine, valine, alanine, leucine, glycine, serine, cysteine, and isoleucine, which may contribute to the observed tyrosinase inhibition activity. Thus, the identified peptides are suitable as tyrosinase inhibitors.

Table 3 Tyrosinase inhibitor peptides of protein hydrolysate MW < 3 kDa from chicken feather meal prepared by pepsin-pancreatin

Fraction	Sequence	Protein name	Accession number	Organism
1	GAGESKC	transthyretin	1666482	*Rattus norvegicus*
	SIFNKGKSIVHKDAW	pkhd1	303225819	*Didelphis virginiana*
	GCGYKPCDPQVIRDRVA	beta spectrin1 protein	218331463	*Sicista betulina*
	AKEKEVTFQSGGPT	Scolopendra 5885.28 Da toxin, partial	212288535	*Scolopendra viridicornis*
	CSARLVNYGYTFGSG	TCR V beta 2-J beta 1.2	1477978	*nigra* *Human*
2	LYFCASSDGLPQDTQYF	T cell receptor beta chain, partial	2894971	*Mus musculus*
	GCYIEGFFATLGGEIALW	rhodopsin, partial	194240743	*Grus Canadensis*
	GNRWLRQAKNG	follistatin	108679	*Bovine*
	M.AAACRCLSLLLLSTCVALLL	Putative pancreatic polypeptide 2	74719120	*Homo sapiens*
	TSMYLCASSSGDREAFFG	T-cell receptor beta chain	5882081	*Homo sapiens*
3	HDDKAAVDAR	Fibrinogen beta chain	120108	*Ceratotherium simum*
	M.SGYGRFHFDQLCHCSFSK	rCG38921	149031319	*Rattus norvegicus*
	NSTMDSLLQLGR	Uncharacterized protein IMPPll, partial	229890311	*Nautilus macromphalus*
	LVNISFGGFIICVFCISIV	Short wavelength sensitive type 1 opsin	226374739	*Larus argentatus*
	ALPGYLK	Glutathione S – transferase P	2143764	*Rat*
4	VATVSLPR	Sarcoplasmic calcium-binding protein, partial	338819392	*Chionoecetes opilio*
	NYMKPKLLYYSNGGH	Fibroblast growth factor 1	122735	*Canis familiaris*
	NRVYVHPF	[Asn1,Val5] angiotensin II	998865	*Amphiuma tridactylum*

4. CONCLUSSION

ACE inhibitory peptides were investigated from 4 kinds of the Thai fruits seeds, which were unripen and ripen papaya seeds, rambutan seeds, longan seeds, and lychee seeds. The crude proteins were hydrolyzed by stimulation of human digestion using gastric enzyme such as pepsin and pancreatin. The hydrolysates were partial purified by ultrafiltration technique with MWCO at 10 and 5 kDa. After fractionated, UF-3 of longan seeds protein hydrolysate (below 5 kDa) had the highest ACE inhibitory activity (IC_{50} with 0.43±0.011 mg protein / ml). Thus, this fraction was subjected to RP-HPLC, the collected peak named P1 - P5 were subjected to LC/MS/MS for identification of the peptides sequences. Mass spectra showed 9 peptide sequences, but only P1-F1, P3-F1, and P3-F4 showed the most inhibitory activity. This was the first study to show the production of antihypertensive peptides by enzymatic hydrolysis of protein from the seeds extract of Thai fruits. The result suggested that some Thai fruits could be a source of peptides that might be a potent source of ACEI bioactive compounds.

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CURRICULUM VITAE

หัวหน้าโครงการ

1. ชื่อ - นามสกุล (ภาษาไทย) นายอภิชาติ กาญจนทัต
   ชื่อ - นามสกุล (ภาษาอังกฤษ) Mr. Aphichart Karnchanatat
2. เลขหมายบัตรประจำตัวประชาชน 3100200084333
3. ตำแหน่งปัจจุบัน (วิชาการ) รองศาสตราจารย์ A-3
   (บริหาร) รองผู้อำนวยการ
   สถาบันวิจัยเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์
   จุฬาลงกรณ์มหาวิทยาลัย
4. หน่วยงานและสถานที่อยู่ที่ติดต่อได้สะดวก
   สถาบันวิจัยเทคโนโลยีชีวภาพและวิศวกรรมพันธุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย อาคารสถาบัน 3 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330
   โทรศัพท์ : 02-218-8078 โทรสาร : 02-253-3543
   โทรศัพท์มือถือ : 081-567-5568 E-mail : Aphichart.K@chula.ac.th
5. ประวัติการศึกษาต้องระบุสถาบันการศึกษา สาขาวิชาและปีที่จบการศึกษา

ชื่อย่อปริญญา	สาขา	สถาบันที่จบ	ปีที่จบ
วท.บ.	เคมี	มหาวิทยาลัยรามคำแหง	2541
วท.ม.	ชีวเคมี	จุฬาลงกรณ์มหาวิทยาลัย	2544
วท.ด.	เทคโนโลยีชีวภาพ	จุฬาลงกรณ์มหาวิทยาลัย	2549

6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการ
   เทคโนโลยีชีวภาพของเอนไซม์ (Enzyme Biotechnology), เคมีของโปรตีน: โครงสร้าง และหน้าที่ (Protein Chemistry: Structure and Function, เคมีของผลิตภัณฑ์ธรรมชาติ (Natural Products), ชีววิธีการฟื้นฟูสภาพแวดล้อมโดยเชื้อรา (fungal Bioremediation)
7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ

7.1 งานวิจัยที่ผ่านมา (ย้อนหลัง 5 ปี)

ลำดับที่	ผู้วิจัยหลัก	หัวข้อเรื่อง	แหล่งทุน	ปีที่ได้	ปีที่เสร็จ
1	รศ.ดร.อภิชาติ กาญจนทัต	เอนไซม์สลายไฟบรินจากเพรียงทราย *Perinereis nuntia* (Fibrinolytic enzyme from *Perinereis nuntia*)	สำนักงานคณะกรรมการวิจัยแห่งชาติ	2555	2556
2	รศ.ดร.อภิชาติ กาญจนทัต	ฤทธิ์ยับยั้งไทโรซิเนสของโปรตีนไฮโดรไลเสตจากเมล็ดผลไม้ไทย (*Tyrosinase* inhibitory activity of the protein hydrolysate from the seeds of Thai fruits)	สำนักงานกองทุนสนับสนุนงานวิจัย (โครงการเชื่อมโยงภาคการผลิตกับงานวิจัย ทุน สกว. - อุตสาหกรรม(MAG Window I)	2555	2557
3	รศ.ดร.อภิชาติ กาญจนทัต	พอลิเมอร์ชีวภาพฉลาดจากพืชสมุนไพรไทยเพื่อการบำบัดโรค (Smart biopolymer from Thai medicinal plants for therapeutic use)	โครงการพัฒนามหาวิทยาลัยวิจัยแห่งชาติ คลัสเตอร์วัสดุขั้นสูง	2553	2556
4	รศ.ดร.อภิชาติ กาญจนทัต (คณะผู้ร่วมวิจัย)	สารออกฤทธิ์ชีวภาพในการป้องกันและรักษาโรคกระดูกพรุนและโรคหลอดเลือดแข็งตัวในผู้สูงอายุ (Bioactive compounds for prevention and treatment of osteoporosis and arthrosclerosis in aging person)	โครงการพัฒนามหาวิทยาลัยวิจัยแห่งชาติ คลัสเตอร์สังคมผู้สูงวัย	2553	2556
5	รศ.ดร.อภิชาติ กาญจนทัต	ฤทธิ์ยับยั้งไทโรซิเนสของโปรตีนไฮโดรไลเสตจากเมล็ดผลไม้ไทย (Tyrosinase inhibitory activity of the protein hydrolysate from the seeds of Thai fruits))	โครงการทุนวิจัยมหาบัณฑิต สกว. สาขาวิทยาศาสตร์และเทคโนโลยี ภายใต้โครงการเชื่อมโยงภาคการผลิตกับงานวิจัย ทุน สกว.-อุตสาหกรรม	2555	2557
6	รศ.ดร.อภิชาติ กาญจนทัต	เรื่อง “การพัฒนาสารต้นแบบสำหรับรักษาโรคจากโปรตีนไฮโดรไลเสต: กรณีศึกษาเมล็ดผลไม้ไทย (Development of therapeutic leads from protein	โครงการทุนช่วยเหลือทางด้านวิจัยวิทยาศาสตร์และเทคโนโลยี (Thailand Toray Science Foundation) ครั้งที่ 20	2556	2557

		hydrolysate: A case study of Thai fruit seeds)	พ.ศ. 2556		
7	รศ.ดร.อภิชาติ กาญจนทัต (คณะผู้ร่วมวิจัย)	ฤทธิ์ยับยั้งไกลเคชั่นของสารชีวภาพจากสมุนไพรไทย (Anti-glycation of biomaterials from Thai herbs)	โครงการส่งเสริมการทำงานวิจัยเชิงลึกในสาขาวิชาที่มีศักยภาพสูง ศูนย์นวัตกรรมสหศาสตร์ โครงการในแผนพัฒนาวิชาการ จุฬาลงกรณ์มหาวิทยาลัย	2556	2557
8	รศ.ดร.อภิชาติ กาญจนทัต (คณะผู้ร่วมวิจัย)	การพัฒนาสารต้นแบบสำหรับการรักษาโรคอัลซัยเมอร์และโรคมะเร็งจากพืชสมุนไทยไทยและราเอนโดไฟต์ที่แยกได้ (Development of therapeutic leads for alzheimer's disease and cancer from Thai medicinal plants and their endophytic fungi)	โครงการวิจัยต่อเนื่อง 7 คลัสเตอร์ คลัสเตอร์สังคมผู้สูงวัย	2556	2558
9	รศ.ดร.อภิชาติ กาญจนทัต	โปรตีนไฮโดรไลเสตจากเมล็ดผลไม้ไทยเพื่อการบำบัดโรค (Protein hydrolysate from Thai fruit seeds for therapeutic use)	โครงการวิจัยต่อเนื่อง 7 คลัสเตอร์ คลัสเตอร์สังคมผู้สูงวัย	2556	2558
10	รศ.ดร.อภิชาติ กาญจนทัต (คณะผู้ร่วมวิจัย)	การการเตรียมโปรตีนไฮโดรไลเสตจากขนไก่ป่นเพื่อการประยุกต์ใช้ในผลิตภัณฑ์เพื่อสุขภาพและเครื่องสำอาง (Preparation of protein hydrolysate from chicken feather meal for applications in health products and cosmetics)	สำนักงานกองทุนสนับสนุนงานวิจัย (โครงการเชื่อมโยงภาคการผลิตกับงานวิจัย ทุน สกว. - อุตสาหกรรม(MAG Window I)	2557	2559
11	รศ.ดร.อภิชาติ กาญจนทัต	การพัฒนาสารต้นแบบสำหรับรักษาโรคมะเร็ง: กรณีศึกษาเพปไทด์ที่มีฤทธิ์ทางชีวภาพจากหอยหวาน (Development of therapeutic leads for cancer treatment: A case study of bioactive peptides from Spotted Babylon)	สำนักงานกองทุนสนับสนุนงานวิจัย (โครงการเชื่อมโยงภาคการผลิตกับงานวิจัย ทุน สกว. - อุตสาหกรรม(MAG Window I)	2557	2559

12	รศ.ดร.อภิชาติ กาญจนทัต (คณะผู้ร่วมวิจัย)	การใช้แอลคาไลน์โปรติเอสในการผลิตโปรตีนไฮโดรไลเสตที่มีฤทธิ์ทางชีวภาพจากขนไก่ป่น (The use of alkaline protease to produced protein hydrolysate with biological activities from chicken feather meal)	สำนักงานกองทุนสนับสนุนงานวิจัย (โครงการเชื่อมโยงภาคการผลิตกับงานวิจัย ทุน สกว. - อุตสาหกรรม(MAG Window I)	2557	2559

7.2 ผลงานวิจัยที่พิมพ์เผยแพร่ (ย้อนหลัง 5 ปี)

7.2.1 Niyomploy, P., Thunyakitpisal, P., **Karnchanatat, A.**, and Sangvanich, P.* (2010) Cell proliferative effect of polyxyloses extracted from the rhizomes of wild tumeric, *Curcuma aromatic* Salisb. *Pharmaceutical Biology* 48: 932-937.

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7.2.4 Tiengburanatam, N., Sangvanich, P., Boonmee, A and **Karnchanatat, A.*** (2010) A novel α-glucosidase inhibitor protein from the rhizomes of *Zingiber ottensii* Valeton. *Applied Biochemistry and Biotechnology* 162: 1938-1951.

7.2.5 Boonmee, A., Srisomsap, C., **Karnchanatat, A.**, and Sangvanich, P.* (2011) An antioxidant protein in *Curcuma comosa* Roxb. rhizomes. *Food Chemistry* 124: 476-480.

7.2.6 Charungchitrak, S., Petsom, A., Sangvanich, P., and **Karnchanatat, A.*** (2011) Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Neilson. *Food Chemistry* 126:1025-1032.

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7.2.13 Boonmee, A., Srisomsap, C., **Karnchanatat, A.**, and Sangvanich P.* Biologically active proteins from *Curcuma comosa* Roxb. Rhizomes. *Journal of Medicinal Plants Research* 5: 5208-5215.

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enzymes in *Pueraria mirifica* cell suspension culture. *Journal of Medicinal Plants Research* 8: 401-407.

7.2.26 Niyomploy, P., Srisomsap, C., Chokchaichamnankit, D., Vinayavekhin, N., **Karnchanatat, A.**, and Sangvanich, P.* (2014) Superoxide dismutase isozyme detection using two-dimensional gel electrophoresis zymograms. *Journal of Pharmaceutical and Biomedical Analysis* 90: 72-77.

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7.2.31 Saisavoey T., Sangtanoo, P., Reamtong, R., and **Karnchanatat, A.*** (2016) Antioxidation and anti-inflammatory effects of defatted rice bran (*Oryza sativa* L.) protein hydrolysate on RAW 264.7 Macrophage cells. *Journal of Food Biochemistry* 40: 731-740.

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tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *Food Science and Biotechnology* (accepted)

7.3 งานวิจัยที่กำลังดำเนินการ

7.3.1 โครงการพัฒนานักวิจัยและงานวิจัยเพื่ออุตสาหกรรม (พวอ.) ระดับปริญญาโท เรื่อง “ฤทธิ์ต้านการอักเสบของเพปไทด์จากขนไก่ป่นในเซลล์เพาะเลี้ยงแมคโครฟาจ RAW 264.7 (Anti-inflammatory activity of peptide from chicken feather meal in Macrophage RAW 264.7)” (หัวหน้าโครงการ) ระยะเวลา 2 ปี (พ.ศ. 2559-2560)

7.3.2 โครงการพัฒนานักวิจัยและงานวิจัยเพื่ออุตสาหกรรม (พวอ.) ระดับปริญญาโท เรื่อง “ฤทธิ์ขจัดอนุมูลอิสระและยับยั้งการเพิ่มจำนวนเซลล์มะเร็งของเพปไทด์จากขนไก่ป่น (Free radical scavenging and antiproliferative of peptide from chicken feather meal)” (หัวหน้าโครงการ) ระยะเวลา 2 ปี (พ.ศ. 2559-2560)

7.3.3 โครงการวิจัย ทุนยุทธศาสตร์การวิจัยเชิงลึก ประจำปีงบประมาณ 2559 งบเชื่อมโยงงานวิจัยระดับคลัสเตอร์ เรื่อง “การเพิ่มมูลค่าผลิตภัณฑ์พลอยได้จากอุตสาหกรรมอาหารทะเลแช่แข็ง: กรณีศึกษากลไกการออกฤทธิ์ต้านอักเสบของเพปไทด์จากกระดูกปลาแซลมอนในเซลล์เพาะเลี้ยงแมคโครฟาจ (RAW 264.7) (Value added products from by-products of frozen seafood processing: A case study of anti-inflammatory mechanisms of peptide from salmon bones in macrophage cell line (RAW 264.7) (หัวหน้าโครงการ) ระยะเวลา 2 ปี (พ.ศ. 2559-2560)