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The protein profile of perch essence and its improvement of metabolic syndrome *in vitro*

Abstract

The objective of this study was to analyze the protein profile and amino acid composition of perch essence processed by high-pressure extraction compared with mackerel, and milkfish. The inhibitory activities of three essences were determined against digestive enzymes relevant to the metabolic syndrome such as the angiotensin-converting enzyme (ACE), pancreatic lipase, α-amylase, and α-glucosidase. The results showed that perch essence exhibits the highest recovery by using the same extraction method for all three fish species. The amounts of all hydrolyzed amino acids, except histidine, were approximately double in perch compared with the other two fish. The perch essence contained the most abundant soluble protein and amino acids composition related to metabolic syndrome. *In vitro* anti-hypertensive, hypoglycemic, and anti-obesity potency of perch essence exhibited the highest among the three fishes. Although the amounts of free amino acids were similar in the three fish species, the molecular weight distribution from gel filtration chromatographic analysis indicated that perch essence contained 32.4% of peptides with a molecular weight of less than 2.3 kDa. This study suggested that naturally occurring bioactive peptides in perch essence might potentially serve as a good source of functional food supplements for metabolic syndrome.

Keywords: amino acid, mackerel, milkfish, perch, peptide, protein

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Introduction

Metabolic syndrome (Mets), including high blood pressure, type II diabetes, obesity, and non-alcoholic fatty liver disease, is a result generally caused by inappropriate lifestyle and eating habits.[1] To alleviate Mets, fish products have anti-Mets effects. Interestingly, it is demonstrated that the fish diet alone has health effects due to the polyunsaturated fatty acid (PUFA).[2] However, previous research demonstrated that lean fish could also have health effects. These results suggested that only fish proteins are beneficial to improve metabolic health other than PUFA.[3] Moreover, aquatic bioactive proteins and peptides have exerted significant hypoglycemic, insulinotropic, hypolipidemic, hypocholesterolemic, hypotensive, and anti-obesity effects in many previous reports. Therefore, fish products are potential therapy candidates for prevention and treatment risk factors.[4] However, herein beneficial effects of various fish species on metabolic dysfunction are required for further comprehensively discussed. Mackerel, milkfish, and perch, rich in biologically active amino acids and protein, are three popular fish species in Taiwan. Among these three species, perch in Taiwan is a traditional food supplement for pregnant women or patients to treat anti-fatigue and obtain quick recovery.[5] Digestive enzymes are important therapeutic targets for the treatment of the metabolic syndrome. The objective of this study was to examine the protein profile and three fish essence products, and the inhibition of the activity of digestive enzymes associated with the development of the metabolic syndrome.

Materials and methods

Sample preparation

The perch (Lates calcarifer), milkfish (Chanos chanos), and mackerel (Scomber japonicus) essences used in this study were produced by Yilan Anyong Lohas Co., Ltd. (Yilan, Taiwan). The fish's internal organs were removed and discarded. The fish samples were then washed to obtain the preliminary fish samples. All these samples were extracted with RO water (w/v=1:1) by high pressure and high temperature, then filtrated with 300 -mesh filter to produce fish essence.

Determination of hydrolyzed amino acid compositions in protein

Hydrolyzed amino acid compositions were analyzed according to the method of ISO 13903:2005 with an amino acid analyzer.[6] Drying sample solution prevents moisture in the sample from affecting the acid concentration of hydrolysis. 500 μg sample powder was acid hydrolyzed in vacuo at 110°C with 6 M HCl containing 0.1% of phenol for 24 hours. The amino acids were separated by ion exchange chromatography and further determined by reacting with ninhydrin. The results were determined by using photometric detection at 570 nm (440 nm for proline). Finally, compare the absorbance intensity of the samples and standard to calculate the concentration, and the unit is mg/100g.

Determination of soluble protein, α-amino group, and amino acid content

The method for detecting soluble protein content was slightly modified from the Folin–Lowry method.[7] Its absorbance was determined at 540 nm, using bovine serum albumin as an analytical standard. The results were converted into soluble protein content. Released α-amino group content, which represents the reactive amino acid, peptide, or protein containing a primary amine was determined with slight modifications through the methods used by Church et al.[8] and Chen & Yang.[9] The reaction was conducted using o-phthaldialdehyde reagent added to the sample. The OPA adducted content was quantified by measuring the absorbance value at 340 nm and using Leu-Gly as a standard. Free amino acid contents were analyzed referring to the improved method reported by Doi et al.[10] Briefly, the sample was mixed with Cd Cl2-ninhydrin reagent and measured the absorbance value at 507 nm using leucine as a standard.

Gel filtration chromatography analysis for protein profile

The sample solution was filtered through a 0.22-μm filter membrane, and 100 μL of the filtrate was used as the injection volume was purified and separated using gel filtration chromatography

(TSKgel GMPWxL Column, Tosoh Bioscience Inc., South San Francisco, CA, USA) and equilibrated with 0.05 M NaNO3. Data analysis was performed using the Viscotek GPC System (1122 pump, 717 Auto-Injector, 270 Dual Detector / Differential Viscometer & Laser Light Scattering Detector, 3580 RI detector, OmniSEC 4.6 Station, Malvern Panalytical Ltd., Malvern, UK).

Angiotensin converting enzyme (ACE) inhibitory activity

The ACE inhibitory activity assay was performed according to the method of Cushman & Cheung.[11] The ACE inhibitory activity was determined by the following equation:

ACE inhibitory activity (%)=[(Ac-As)(Ac-AB)]100%

Ac is the absorbance of the buffer (control).

As is the absorbance of the reaction mixture (sample)

AB is the absorbance when the stop solution was added before the reaction occurred (blank).

Pancreatic lipase inhibitory activity

The ability of the compounds to inhibit pancreatic lipase was measured using the method previously reported by Liu et al.[12] and Glisan et al.[13] with some modifications. Lipase inhibitory rate was calculated as follows:

Lipase inhibitory rate (%) = (1 - Ab/Aa) × 100

Aa represented the lipase activity without any inhibitor.

Ab represented the lipase activity in the presence of the sample.

Assay for α-amylase and α-glucosidase inhibitory activity

The a-amylase inhibition assay was adopted from Hasenah et al.[14] The α-glucosidase inhibitory activity assay was performed according to the method by Oki et al.[15] The inhibitory activity was calculated as follows:

Inhibitory Activity (%) = (AC -(AS - ACB)/ AC) × 100%

AC represented the α-glucosidase activity without any inhibitor.

AS represented the α-glucosidase activity in the presence of the sample.

ACB represented the α-glucosidase activity without any enzyme.

Statistical analysis of data

The data obtained in this study were statistically analyzed using SAS software (Statistical Analysis System, SAS; Version 9.4 TSIM5, SAS Institute Inc., Cary, NC, U. S. A.). The values of the experimental results were presented as mean ± SEM. The experimental data were analyzed by one-way ANOVA to determine the differences between groups, and Duncan's multiple range test was used to compare differences between groups.

Results and discussion

From the results of soluble protein, released α-amino group, and free amino acid analysis in Table 1, the constituents of three essences were mainly protein which was determined by colorimetric method. Perch in particular contains the highest amount of protein, nearly twice as much as milkfish and mackerel by using the same extraction method. Three essences contained similar released α-amino group and free amino acid content. In addition to histidine, perch protein contains almost twice as much amino acid as milkfish and mackerel (Table 2). The hydrolyzed amino acid composition of protein in perch essences is mainly glycine, glutamic acid, alanine, proline, arginine, aspartic acid, and lysine. Fish proteins usually contain most of the essential amino acids and have a very high content of the essential amino acids lysine and leucine.[3] similar to this study. Of the nonessential amino acids, aspartic acid, glutamic acid, and alanine are present in high amounts in fish protein.[3] Interestingly, perch concentrate contained abundant glycine as shown in Table 2. Glycine is required for multiple metabolic pathways in addition to being a building block for proteins and as a conditionally essential amino acid associated with metabolic syndrome.[16] Therefore, supplementation of abundant glycine content in perch essence could be recognized to improve mild deficiency in glycine and may participate in the Mets.

Table 1 Soluble protein, α-amino group and free amino acid concentration in fish essence

	Concentration(mg/mL)		
Essence	**Soluble protein**	**α-Amino group**	**Free amino acid**
Perch	66.80±0.56a	10.63±0.09c	2.97±0.02c
Milk fish	36.32±0.67b	14.33±0.18a	6.62±0.26a
Mackerel	32.76±0.81c	12.49±0.81b	4.92±0.45b

Different superscripts in the same column indicate significant different ($p < 0.05$) between samples

Table 2 Major hydrolyzed amino acid compositions of three fish essences

	Concentration (mg/100g)		
Amino acid	**Perch**	**Milkfish**	**Mackerel**
Aspartic acid	552	313	328
Glutamic acid	934	528	551
Serine	233	155	175
Histidine	85	753	438
Glycine	1570	766	502
Threonine	241	119	143
Arginine	590	315	263
Alanine	800	388	318
Valine	223	130	149
Methionine	150	86	81
Phenylalanine	197	114	98
Leucine	298	196	227
Isoleucine	142	100	98
Lysine	403	271	288
Proline	779	420	235
Total	7197	4654	3917

Among the three fish species selected for the study, perch had higher inhibitory activity for ACE, α-amylase, α-glucosidase, and lipase Table 3 than milkfish and mackerel. The results indirectly suggested that perch essences were the most effective in lowering blood pressure, blood sugar, and lipids.

Table 3 Four digestive enzyme inhibitory activity (%) of fish essence

	Inhibition (%)		
Enzyme	**Perch**	**Milkfish**	**Mackerel**
ACE	85.92±0.22a	68.14±2.03c	80.79±4.14b
α-Amylase	99.37±0.89a	86.18±2.71b	88.80±0.50b
α-Glucosidase	97.41±4.99a	86.37±5.73b	87.07±2.57b
Pancreatic lipase	83.05±0.25a	73.67±0.25b	62.67±0.90c

Different superscripts in the same row indicate significant different ($p < 0.05$) between samples.

Perch essence processed from extraction contained about 84 mg/mL of protein. It was also found neither lipid nor cholesterol was detected in the essences (data not shown). As most of the fish proteins that are effective anti-Mets are bio-peptides with molecular weights below 1000.[4,17] To investigate why perch has the highest activity, this study further analyzed the molecular weight distribution of perch essences using gel filtration chromatography. The perch essences with molecular weight less than 1.2 kDa accounted for the total protein was 22.6% from the results of protein profile analysis in Table 4. The perch essences contained nearly 32.4% peptides with a molecular weight of 240-2300 Da. Many research indicated that bioactive fish proteins or peptides tend to be present as oligo-peptides with low molecular weight and contain 2 to 20 amino acid residues (near 3 kDa).[3,4] This result suggested that although the majority of molecules of perch essences are proteins determined by the colorimetric method as in Table 1, the high inhibitory activity of perch essences on digestive enzymes might be related to the presence of these smaller molecular proteins or peptides with a molecular weight of less than 2.3 kDa. The metabolic health effects of a fish protein depend on several factors, such as the amino acid composition of the protein, the steric structure of the protein, and chemical changes of the protein during processing. The bioactivity of peptides is associated with the presence of various amino acid sequences.[3,17] Our results showed that various amino acid sequences in these three fish-derived essences after being processed by high-pressure extraction are capable of reducing hypertension by inhibiting angiotensin-converting enzymes and the blood glucose and lipid metabolism can be well regulated. Moreover, perch essence exhibited prominent activity due to its bioactive peptide.

Table 4 Molecular weight distribution of perch essence by gel filtration chromatography

Molecular weight	Percentage (%)	Description
>2300	62.90%	protein
2300-1200	14.50%	peptide
1199-580	10.70%	oligopeptide
579-240	7.90%	
239-189	1.30%	
<189	2.70%	free amino acid
Average	5843 (Da)	

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None.

Conflicts of interest

The authors declare no conflicts of interest.

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