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Flavored-functional protein hydrolysates from enzymatic hydrolysis of dried squid by-products: Effect of drying method

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ABSTRACT

Dried squid head is an abundant–low value by product from dried squid snack industry. The head is consisted of high protein content with the abundant sweet-umami amino acid, glutamic acid (7.45 mg/100 mg). The major volatile compounds found are trimethylamine and toluene along with the 15 important compounds exhibited dried squid flavor. Enzymatic hydrolysis with Flavourzyme® was performed at the optimal pH and temperature in order to produce flavored-functional protein hydrolysate. The supernatant was collected at 0, 30, 60, 90, 120, 150, 180 and 210 min of hydrolysis. The sweet-umami hydrolysate solution with the highest liking score at 6.64 was obtained after 180 min of hydrolysis. This sample was subjected to dry using freeze drying and foam-mat drying and investigated the changes of flavor compounds and functional properties. The freeze dried sample had umami taste and light brown color, and possessed the various types of volatile compounds. It contained protein at 76.42%, and EAI at 22.00, while the foam-mat dried sample had the highest ESI (15.27) and FS (29.87). The antioxidant property of the freeze dried sample (ABTS scavenging activity at 14.77 mg/g, FRAP scavenging activity at 5.93 mmol Fe_2SO_4 $7H_2O$/g and DPPH scavenging activity at 19.51%, respectively) was higher than that of the foam-mat dried sample. Hence, it could be used as flavored-functional ingredient in foods.

1. Introduction

Annually, Thailand generates high revenue from the export of seafood products. In 2015, seafood products showed the total export value of 1,537,710 tones accounted for 189,260 million baht (Fisheries Foreign Affairs Division, 2015). The large income was received; however, there was a lot of by-product generated during processing, such as fish head, fish bone, fish skin, shrimp shell and squid head. As a result, various researches interested and tried to increase the value of those abundant wastes using various methods.

Enzymatic hydrolysis is one of the alternative methods that can be used for producing high value-functional peptide from the seafood by-products. There are some reports revealed the producing method of peptide with angiotensin-converting enzyme and antimicrobial property using enzymatic hydrolysis (Chen, Wanga, Zhongb, Wua, & Xia, 2012; Cheung & Li-Chan, 2014). Antioxidant hydrolysate was produced from flying squid by-products using papain hydrolysis with the DPPH radical scavenging activity of 74.25% (Fang, Xie, Chen, Yu, & J. Chen, 2012). Moreover, an application of enzymatic hydrolysis for producing functional peptide were also reported as flavor peptide, emulsifying agent, foaming agent, thickening agent and stabilizing agent (Liu et al., 2014). For flavor peptide, the research of Peinado, Koutsidis, and Ames (2016) found that enzymatic hydrolysis of fish by-products produced seafood flavor peptide by using Flavouzyme®. They found that peptide was composed of various amino acids such as lysine, leucine, glutamic acid and alanine. Moreover, by-products of seaweed (*Gracilaria* sp.) were used to produce the seafood-like flavor using bromelain hydrolysis (10% bromelain for 3 h). Arginine, lysine, and leucine, were the top three free amino acids found in the flavored hydrolysate (Laohakunjit, Selamassakul, &

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Kerdchoechuen, 2014). The other alternative method that could enhance flavor of protein is heat treatment. Heat affected flavor of peptide by increasing main volatile compound in cooked seafood including 1-octen-3-octenol or 1-hepten-4-octenol (Peinado et al., 2016). The increase of total volatile compound of squid was reported after drying (Deng, Luo, Wang, & Zhao, 2015). Moreover, treating protein solutions with heat not only hydrolyzed the protein, but also affected the type and concentration of peptides released (Arrutia, Puente, Riera, Menendez, & Gonzalez, 2016). The appropriate condition for enzymatic hydrolysis creates the reaction which resulted in functional peptide (Vijaykrishnaraj, Roopa, & Prabhasankar, 2016). In Thailand, there was an abundant by-product from seafood industry. Dried squid head is one of the major by-product from dried squid snack industry. In order to increase the by-product value, this research was selected dried squid head as raw material for producing flavored-functional peptide and characterized their property including proximate compositions, chemical, physical and sensory characteristics. The effect of drying method on the amount and type of odor and flavored compound and functional property of the resulting protein hydrolysate was also studied to obtain the novel functional-flavored peptide suited for utilizing as food ingredients.

2. Materials and methods

Dried squid head, the by-products from squid snack industry, was supported by T Thai Snack Foods Co. Ltd., Bangkok, Thailand and stored at −18 °C until use. Flavourzyme® 1000L was supported by Brenntag Ingredient (Thailand) Public Co Ltd, Bangkok, Thailand and stored at 4 °C prior to use. All chemical reagents used in this research are analytical grades.

2.1. Determination of dried squid head properties

Total amino acid composition of dried squid head was determined using high-performance liquid chromatography column (HPLC, 1100 series; Agilent Technologies Inc., Santa Clara, CA, USA) based on the method of AOAC (1995). The sample (1 μl) was injected on a Zorbax 80A C18 column at 40 °C and detected at 338 and 262 nm. The amino acid compositions are expressed in term of mg per 100 mg amino acid in dried squid head.

Proximate compositions of dried squid head were determined according to the method of AOAC (2000).

Volatile compounds were determined based on the method of Benet et al. (2015). One-gram of the homogenised samples were placed into a 20 mL glass vial, capped with a Silicone-PTFE septum and transferred into a CTC SPME AutoSampler (CTC Analytics AG, Zwingen, Switzerland). The SPME fibers (a 50/30 μm DVB/Cacboxen™/PDMS StableFlex™, Supelco: 57328-U) were exposed to the headspace of the vial for 30 min at 40 °C and the volatiles were desorbed in the injection port of the chromatograph for 14 min at 250 °C in split-less mode. An empty vial was used as a blank sampler to clean the column and the fiber before each sample run. Gas chromatography and mass spectrometry (GC-MS) analysis: The analysis were performed with an Agilent 6890 gas chromatograph coupled to a 5973 N mass selective detector from Agilent (Agilent, Palo Alto, USA). The separation of volatiles was performed using an Agilent 19091N-133 HP-INNOWax, 0.25 mm × 30 m * 0.25 μm capillary column and helium was used as the carrier gas. The oven program includes an initial temperature of 40 °C, and a program rate of 6 °C/min up to 250 °C. The mass spectrometer (MS) transfer line temperature was held at 250 °C. Electronic impact at 70 eV was used to obtain the mass spectra. Volatile compounds were identified comparing their mass spectra with references from several commercial libraries databases NIST 08 library (NIST 08, version 2.0, Gaithersburg, USA) and Wiley (Willey & Sons Inc., Germany).

2.2. Production of flavored-functional protein hydrolysate using commercial enzymatic hydrolysis

In this experiment, enzymatic hydrolysis with the commercial protease, Flavourzyme®, was performed in order to produce flavored-functional protein hydrolysate. The mixture of dried squid head and water was prepared by mixing the head and water at the ratio of 1:2 using Waring blender (11,000 rpm for 1 min), heating under high pressure at 121 °C, and hydrolyzing with 1% (w/w) Flavourzyme® under the optimal pH and temperature (Fang, Xie, Chen, Yu, & Chen, 2012). The reaction time was varied at 0, 30, 60, 90, 120, 150, 180 and 210 min using CRD design. The supernatant was collected and heated at 90 °C for 15 min in order to inactivate the enzyme (Muzaifa, Safriani, & Zakaria, 2012); consequently, the properties including protein content (mg/ml), salt content (%), total soluble solid (°Brix), pH, degree of hydrolysis (DH, %), nitrogen solubility index (NSI, %), color (CIE L*, a*, b*), and sensory characteristics, was performed as described below.

Protein content of the obtained hydrolysate solution was determined on according to the method of AOAC (2000). The salt content in samples was determined using the method of Hjalmarsson, Park, and Kristbergsson (2007). Total soluble solids (°Brix) were determined by refractive index with a refractometer (Carl Zeiss IMT Corp. Brighton, Michigan, USA) based on the method of Hjalmarsson et al. (2007). The pH value of all samples was measured with a pH meter (Sartorius, Docu-pH-Meter, USA) using 50 mL of sample (Hjalmarsson et al., 2007). The color of supernatant was determined using the chromaticity instrument (CM-3500d Minolta Co., Japan). A white standard board was used for calibration.

Degree of hydrolysis, DH (%) was defined as the percentage of number of peptide bonds or free amino groups cleaved from protein compared with total number of peptide bonds in substrate and can be calculated from spectrophotometer readings of the serine standard and the test sample. The OPA method was used to investigate DH of all samples, according to the method of Nielsen, Petersen, and Dambmann (2001).

Nitrogen solution Index (%NSI) was performed based on the method of Gimenez, Aleman, Montero, & Guillen, 2009. The protein hydrolysates (0.5 g) were mixed in 50 ml of 0.1 M sodium chloride (pH7) by using magnetic stirrer for 1 h at room temperature. The supernatant was collected by filtration and determined for protein content. %NSI was calculated as follow:

$$NSI\ (\%) = (A/B) \times 100$$

Where

A is protein content in the supernatant
B is total protein content in the sample.

The color values were expressed as L* (whiteness/darkness), a* (redness/greenness) and b* (yellowness/blueness). An average value of five replications was reported (CIE, 1986). All experimental tests were made in triplicate.

The sensory properties of the dried squid head hydrolysate were investigated. Fifty panelists, experienced in sensory evaluation of foods were asked to evaluate the samples for overall liking score using a nine point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely) for each sensory attribute. Then, the collected data was analyzed using one way ANOVA at $p \leq 0.05$.

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