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Processed flavors derived from combined bromelain hydrolyzed jellyfish protein hydrolysate, reducing sugars and arginine

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ABSTRACT

Food flavor compounds can be thermally produced by the reaction of reducing sugar and amino acid at specific conditions of time, temperature, pH, and water content. The pathways of Maillard reaction, Amadori rearrangement, Strecker degradation, and Schiff bases contribute to flavor and color. To date, no report on jellyfish by-products has been involved in generating food flavors. The objectives of this study were to investigate hydrolysis factors (enzyme concentration and hydrolysis time) of jellyfish by-products on qualities of enzymatic bromelain-jellyfish protein hydrolysate (eb-JPH). The characterization of volatiles obtained from selected eb-JPH reacted with mixed sugars (glucose and ribose), and arginine was by GC-MS. Results showed that the protein of dried jellyfish by-product was 68.8%. The reaction for producing eb-JPH was bromelain 20% for 18 h at pH 6 and 50°C and generated the highest yield and degree of hydrolysis at 76.09% and 80.94%. The volatile flavors developed by the reaction of eb-JPH, sugars (glucose and ribose), and arginine prepared in the ratio of 0.1:1:0.08:0.08 (w/w) heated at 95±2°C for 2 h yielded 13 volatile compounds in which benzaldehyde and methyl pyrazines were significant contributors to the meat-like flavor.

Keywords: process flavor; maillard reaction; bromelain; jellyfish

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1. INTRODUCTION

Processed flavors are thermally produced via the Maillard reaction by reducing sugar and free amino acid. During the Maillard process, the dicarbonyl compounds are derived by the Strecker degradation, thus generating various color and flavor compounds. Factors affecting flavor production are the type and concentration of reducing sugar and amino acid, specific conditions of temperature, pH, moisture or water content, duration time, and type of thermal process used (Chiang et al., 2019; BeMiller and Huber, 2008). To date, sources of amino acid in the form of protein hydrolysate obtained from different raw materials have been reported, including beef bone (Chiang et al., 2019), beef (Zou et al., 2019), scallop mantle (Han et al., 2018), and soybean (Fadel et al., 2018). By-products have also been involved in the flavor production, such as seaweed by-products (Laohakunjit et al., 2014), fish by-products (Peinado et al., 2016), and squid by-products (Sukkhown et al., 2018). These studied flavors have received much attention due to the resulting uniqueness of aroma or flavor, the process's controllability, and flavor quality.

To date, producing enzymatic protein hydrolysate has received much attention. One of the commercial enzymes

used in hydrolysis is bromelain (Silaprueng et al., 2015; Laohakunjit et al., 2014; Liu and Chiang, 2008; Sonklin et al., 2011). Bromelain is a cysteine endoproteinase extracted from the stem of pineapple fruit, offering a broad range of pH stability (pH 4-8) (Laohakunjit et al., 2014). Apart from the enzyme, the characteristic of protein source should be rich in protein or by-products. In this study, jellyfish by-products, a waste from manufacturing and low in feed meal prices, were chosen. Research on the quality of jellyfish protein hydrolysate (JPH) has been reported by Thumthanaruk and Lueyot (2014); Silaprueng et al. (2015); and Kromfang et al. (2015). The functional properties of JPH are foaming, emulsifying, and antioxidant activity, which may differ in terms of value, depending on the sample used and hydrolysis conditions. Klaiwong et al. (2014) and Kromfang et al. (2015) also reported amino acid contents in jellyfish, which definitely can be a source of amino acids for flavor production. However, processed flavor developed from by-products of jellyfish hydrolyzed by bromelain (eb-JPH) and sugars has not been characterized. Hence, in this study, the objectives were to investigate the effect of bromelain concentration and hydrolysis time on qualities (color, pH, soluble protein, degree of hydrolysis (DH), and yield) of eb-JPH. The processed volatiles obtained from selected eb-JPH with the highest DH reacted with mixed sugars (glucose and ribose), and arginine was analyzed by GC-MS.

2. MATERIALS AND METHODS

2.1 Preparation of dried jellyfish by-product

The preparation of desalted jellyfish was carried out by the modified method described by Thumthanaruk and Lueyot (2014) and Charoenchokpanich et al. (2020). The wash step was performed in triplicate. Briefly, salted by-products of jellyfish (*Lobonema smithii*) were washed by a jellyfish washing machine for 30 min. The soluble salt content was 0%, as measured by a salinity refractometer (Master-S/Mill M, ATAGO®, Japan). The washed by-products were drained for 30 min, packed in polyethylene bags, and transferred to the Department of Agro-Industrial, Food, and Environmental Technology. The by-product samples were dried in a hot air oven (G 01350, Bluem, USA) at 50°C for 24 h, ground to 35, 60, 80, and 100 mesh, and stored in sealed polyethylene bags at room temperature until used. Proximate compositions of dried jellyfish were determined according to the method of AOAC (2000).

2.2 Production of dried jellyfish protein hydrolysate by water hydrolysis (w-JPH)

Each size (35, 60, 80, and 100 mesh) of dried jellyfish powder was hydrolyzed by water incubated at 50°C for 6 h in an incubator (WIS-20R, WiseCube, Korea). The dried jellyfish powder ratio to water used was 1:20 (w/v) with 5 mL and performed in triplicate. The sample was then centrifuged using a centrifuge (Suprema 21, Tomy, Japan) at 9000 rpm at 4°C for 10 min and filtered. The supernatant was subjected to a freeze dryer (Alpha1-4 LSCplus, Martn Christ, Germany) operating at -50°C, drying at 40°C and 0.1 mbar for 24 h. The freeze-dried w-JPH was stored in sealed polyethylene bags at room temperature until used.

2.3 Production of jellyfish protein hydrolysate hydrolyzed by bromelain enzyme

The procedure of bromelain hydrolyzed jellyfish protein hydrolysate was followed by the method of Silaprueng et al. (2015). Briefly, the dried jellyfish powder (from section 2.1) with 100 mesh size was dispersed in distilled water at 1:20 (w/v). The samples were incubated at 95°C for 15 min and then adjusted to a pH of 6.0 with 1N HCl. The studied factors were bromelain concentration having the activity of 200 gelatin dissolving units (GDU)/g (10, 15, and 20% w/w), hydrolysis times (6, 12, and 18 h) at pH 6.0 and 50°C. The reaction mixture was prepared for 5 mL and performed in triplicate. The enzymatic reaction was terminated by heating at 95°C for 15 min. The hydrolysate was centrifuged at 6,000 rpm for 15 min using a high-speed refrigerated centrifuge. The supernatant was freeze-dried using a freeze-dryer with a control condition described in section 2.2 and kept in a seal polypropylene bag until analysis.

2.4 Production of thermally processed flavor powder

The production of thermally processed flavor was performed in duplicate and modified from the method of Laohakunjit et al. (2014). The reaction mixture of the eb-JPH powder sample with the highest DH value (0.5 g), glucose (0.3 g), ribose (0.2 g), and distilled water 1 mL was heated at 95±2°C for 2 h in a water bath. For the addition of amino acid, the reaction mixture of eb-JPH powder (0.5 g), glucose (0.3 g), ribose (0.15 g), arginine (0.05 g), and distilled water 1 mL was formulated and heated as previously described. After the thermal incubation, all samples were freeze-dried by a freeze dryer. The freeze-dried sample was kept in a seal polypropylene bag until analysis.

2.5 Analysis

2.5.1 Proximate analysis

The contents of protein, fat, ash and moisture in the dried jellyfish by-products were performed in triplicate according to the AOAC method (AOAC, 2000).

2.5.2 Degree of hydrolysis

The degree of hydrolysis (DH) was measured by the method of Benjakul and Morrissey (1997). A quantity of 125 µL of JPHs (w-JPHs and eb-JPH) was mixed with 2 mL of 0.2 M phosphate buffer (pH 8.2) and 1 mL of 0.01% TNBS solution. The solution was incubated at 50°C for 30 min in a temperature-controlled water bath (WNB7, Memmert, Germany) in the dark. A quantity of 2 mL of 0.1 M sodium sulfite was added to stop the reaction. After cooling at room temperature for 15 min, the absorbance was measured at 420 nm. The percentage of DH was calculated as follows:

$$DH\ (\%) = [(L-L_0)/L_{max}-L_0)] \quad (1)$$

where L is the amount of α-amino groups of the hydrolysate sample, L_0 is the amount of α-amino groups in the original substrate (blank), and L_{max} is the total α-amino groups in the sample hydrolyzed by 6N HCl at 100°C for 24 h.

2.5.3 NaCl determination

The salt content of eb-JPHs was measured using the modified titration method (AOAC, 2000). The reaction mixture of a sample (1 g), 25 mL of 0.1 N silver nitrate ($AgNO_3$), and 10 mL of 68% nitric acid (HNO_3) were boiled for 10 min. After cooling at room temperature for 15 min, distilled water (50 mL) and 5% ferric alum indicator (5 mL) were added to the sample. The sample was titrated with 0.1 N potassium thiocyanate (KSCN) until the solution developed orange color. The salt content (%) was calculated by Equation (2).

$$Salt\ (\%) = \frac{(V_1 \cdot N_1) - (V_2 \cdot N_2)}{M} \cdot 100 \quad (2)$$

where V_1 = volume of $AgNO_3$ (mL), V_2 = volume of KSCN (mL), N_1 = concentration of $AgNO_3$ (N), N_2 = concentration of KSCN (N), and M = weight of sample (g).

2.5.4 pH

The eb-JPH samples (5 g) were mixed with 45 mL of distilled water. The mixture was homogenized for 3 min and then filtered. The pH of the filtrate was measured using a pH meter (pH 700, Eutech, Singapore).

2.5.5 Color measurement

The color of JPH and eb-JPH powders was measured using a colorimeter (Color QUEST 45/0, HunterLab, USA). The sample of 10 g was filled in the glass receptacle and measured three times by rotating at different angles. The results of the CIELAB color parameters were expressed as L^* (lightness 0-100), a^* (+redness, – greenness), b^* (+yellowness, -blueness).

2.5.6 Yield

The freeze-dried samples obtained from section 2.3 were weighed using the electric balance. The yield was calculated from the weight of jellyfish by-product powder and weight of freeze-dried eb-JPH powder by Equation (3).

$$Yield\ (\%) = \frac{M_2}{M_1} \cdot 100 \quad (3)$$

where M_1 = weight of jellyfish by-product powder and M_2 = weight of freeze-dried eb-JPH powder.

2.5.7 Determination of processed flavor by gas chromatography-mass spectrophotometry (GC-MS)

The volatile compounds developed from process flavor were characterized by the combination of dynamic headspace and thermal desorption-gas chromatography-mass spectrometry (DH-TD-GC/MS) (Kuraya et al., 2018). Briefly,1 mL of the sample was packed and septum-sealed in a 27-mL gas-tight vial. The volatiles were aspirated by a minipump and adsorbed to Tenax TA (60/80 mesh, 130 mg) for 10 min at 60°C after passing air through the vial. The GC/MS (QP-2010 Plus; Shimadzu Co., Kyoto, Japan) equipped with a TD-20 thermal desorption system (Shimadzu, Japan) was used in this analysis. GC/MS analyses were performed using a DB-WAX column of 60-m length, 0.32-mm ID, and 0.5-µm thickness (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC oven temperature program was as follows: 40°C held for 3 min, increased by 3°C per min to 130°C, increased by 5°C per min to 240°C, and held for 5 min. The GC was interfaced with the temperatures of the injector and ionization source, maintained at 240°C. The mass range scanned was 20-400 amu. The volatile components were quantified based on peak area measurements using 'Shimadzu's GC/MS solution software, version 4.2, and identified by comparing their retention indices and mass fragmentation patterns with MS libraries (NIST05 and FFNSC Library ver. 1.2; Shimadzu Co., Kyoto, Japan). The linear RIs were determined using a homologous series of n-alkanes (C8-C24).

2.6 Statistic analysis

The experiments were performed in triplicate, but the volatile compound determination was performed in duplicate. The software SPSS (SPSS 22.0 for Windows, SPSS Inc, Chicago, IL USA) was used to determine the analysis of variance (ANOVA) and Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Quality of dried jellyfish by-product

The raw material of dried jellyfish by-products had protein, fat, ash, and moisture of 68.8%, 3.18%, 9.11%, and 5.27%, respectively. The results suggested that jellyfish by-products can be further used for producing protein hydrolysate for the benefit of protein content. The results were slightly different from the works of Silaprueng et al. (2015) and Charoenchokpanich et al. (2020). Silaprueng et al. (2015) used the umbrella portion, not by-products, and reported that dried white jellyfish (*Lobonema smithii*) had protein, fat, moisture, and ash of 75.09%, 5.16%, 9.66%, and 8.56%, respectively. By using the same washing jellyfish machine, Charoenchokpanich et al. (2020) who reported the protein, ash, fat, and moisture of desalted jellyfish (washed 2 cycles) of 4.79±0.07%, 1.39±0.17%, 0.95±0.04%, and 91.86±0.06%, respectively. Previous reports also showed that most of the jellyfish protein was collagen (Hsieh et al., 2001; Klaiwong et al., 2014). Then, the use of jellyfish by-products as a source of protein was selected for generating protein hydrolysate.

3.2 Effect of size of dried jellyfish powder on DH of water hydrolyzed jellyfish protein hydrolysate (w-JPH)

The size of dried jellyfish powders was varied to facilitate hydrolysis. Results showed that the smallest size (particles passing through 100-mesh sieve) had a lighter brown color than the others (Figure 1). After being subjected to water hydrolysis at 50°C for 6 h, the results revealed that the finest dried jellyfish had the highest degree of hydrolysis at 24.02% (data not shown). The fine particle of dried jellyfish powder increased surface area; it increased the degree of hydrolysis. Colors of powders are affected by the powder fineness. When the white light illuminates, the powder's color becomes light closer to white (Radjenović et al., 2018). According to the work of Klaiwong et al. (2014), the collagen protein's denaturation temperature in jellyfish is at least 70°C. Hence, the condition of 50°C for 6 h used for hydrolysis might not be sufficient to hydrolyze major collagen protein. However, the temperature of 50°C was suitable for bromelain to function in the next experiment. Then, the temperature chosen for hydrolysis was used at 50°C in this study.

3.3 Effect of bromelain concentration and hydrolysis time on yield, DH, NaCl, pH, and color of the eb-JPH powder

Bromelain hydrolyzes peptide bonds of jellyfish protein, thereby yielding short-chain and free amino acids depending on the hydrolysis condition. Results showed that increased enzyme concentration increased the yield and DH. When the reaction mixture was performed at the same enzyme concentration, increased hydrolysis time increased yield and DH. The hydrolysis condition at bromelain concentration of 20% for 18 h generated the highest yield and degree of hydrolysis of 76.09% and 80.94%, respectively. During the hydrolysis, the bromelain enzyme can cleave peptide bonds of collagen jellyfish into shorter chains and smaller molecules. However, the value of the degree of hydrolysis and yield may be affected by the degree of jellyfish protein denaturation. The same hydrolysis condition showed higher DH than those of Silaprueng et al. (2015) that the effect of particle size was not investigated. The results confirmed that the reduced size of jellyfish sample increased the surface area where bromelain can hydrolyze the collagen protein's peptide bonds efficiently, thereby increasing the degree of hydrolysis results. Among eb-JPH samples, the values of %NaCl, pH, and color measurement were not significantly different (p>0.05) (Table 1). Figure 2 shows that all freeze-dried eb-JPH powders had the shade of white to light brown color.

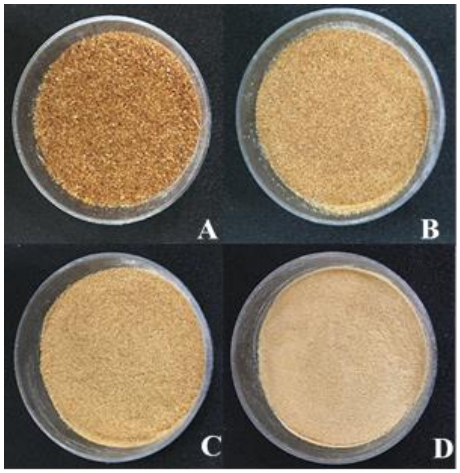

Figure 1. Appearances of dried jellyfish with particles passing through (A) 35, (B) 60, (C) 80, and (D) 100 mesh sieve

Table 1. DH, yield, NaCl, pH, and color of JPH and eb-JPH at different bromelain concentrations (10, 15, and 20% w/w) and duration times (6, 12, and 18 h)

Concentration (%)	Time (h)	DH* (%)	Yield* (%)	NaCl (%)NS	pHNS	ColorNS		
						L*	a*	b*
0	6	28.73^{a}±0.16	46.27^{a}±0.47	2.22	5.48	84.11	0.81	12.58
	12	38.30^{b}±0.10	50.84^{c}±0.49	2.07	5.46	85.17	0.83	12.78
	18	48.27^{c}±0.97	51.68^{d}±0.40	2.05	5.43	86.02	0.70	13.0
10	6	71.99^{d}±0.14	50.67^{b}±0.30	1.50	5.72	85.14	0.94	12.71
	12	76.26^{f}±0.07	68.89^{e}±0.47	1.66	5.65	83.51	1.52	13.70
	18	78.82^{h}±0.22	70.61^{f}±0.39	1.63	5.62	81.20	0.82	12.60
15	6	75.56^{e}±0.23	70.46^{f}±0.50	1.64	5.74	82.77	0.90	12.71
	12	80.02^{i}±0.28	73.47^{h}±0.47	1.62	5.68	85.14	0.81	12.75
	18	80.30^{j}±0.22	75.52^{j}±0.49	1.70	5.59	87.40	0.33	12.59
20	6	76.60^{g}±0.10	71.82^{g}±0.40	1.70	5.66	83.52	0.32	10.79
	12	80.09^{i}±0.20	74.86^{i}±0.48	1.56	5.61	85.99	0.65	12.96
	18	80.94^{k}±0.27	76.09^{k}±0.50	1.56	5.55	86.78	0.82	14.03

Note: Different lowercase letters within the same column indicate significant differences (p<0.05)
NS refers to no significant difference (p>0.05)

3.4 Volatile compounds derived from process flavor

In this study, the processed flavor was thermally generated by mixing eb-JPH (from 20% bromelain at 18 h of hydrolysis) and glucose and ribose sugars (JRG). Results of JGR showed 11 volatile compounds that were classified as an aldehyde (hexanal, 3-methyl butanal, nonanal, furfural, benzaldehyde), alcohol (2-butanol), ketone (2-butanone, 1-hydroxy-2-propanone, 1-methyl-2-pyrrolidinone), and acid (propanoic acid, 3-methyl butanoic acid). The mixed odors described were fishy, sweet, nutty, almond, and fatty. With this description, no exact flavor perception was classified. As is generally known, the cascade Maillard reaction is a crucial process for producing color and flavor compounds. At the beginning step, the compounds of furfural, furanone derivatives, hydroxy ketones, and dicarbonyl compounds are formed and subsequently reacted with other amines, amino acids, aldehydes, hydrogen sulfide, and ammonia through the Amadori rearrangement, Strecker degradation, and Schiff bases pathways, leading to the formation of melanoidins and high molecular weight of brown polymers. The Strecker degradation products occur through the reaction between carbonyl compounds and amino acid, give Strecker aldehyde and α-aminoketones, which are indicators of meat flavor heterocyclic compounds (Chiang et al., 2019; BeMiller and Huber, 2008). Then, the addition of arginine to the previous reaction delivered a better volatile flavor profile. The solution mixture of eb-JPH, mixed sugars of glucose and ribose, and arginine (JGRA) gave 13 flavor compounds. Three compounds (pyrazine, methyl pyrazine, and 2-furan methanol) were newly found, but one compound of 3-methyl butanoic acid was missing (Table 2). The reduction of furfural obtained when the reduced content of ribose sugar was applied.

Adding arginine amino acid developed benzaldehyde, an indicator of meat flavors, pyrazine, methyl- pyrazine, and furfural indicators of roasted flavors (Shimoda and Shibamoto, 1990). The different flavor profiles are affected by the type and concentration of reducing sugars and amino acids and thermal conditions. The results were different from Laohakunkjit et al. (2014) and Peinado et al. (2016). To generate seafood flavor, Laohakunjit et al. (2014) varied a mixture of glucose, ribose, taurine, arginine, alanine, glycine, and paste-like enzymatic bromelain seaweed protein hydrolysate (eb-SWPH) at 2:1:2:2:2:8:15 g, adjusted to 5.5 with 1 N HCl, and heated the solution at 95° C for 120 min. Results showed that reducing sugars (glucose and/or ribose) and amino acids (glycine, alanine, and arginine) were used as reactants. The addition of taurine enhanced the seafood profile flavor. The compounds indicating seafood flavor were 2,5-dimethyl pyrazine, 2,3-dimethyl pyrazine, trimethyl pyrazine, benzaldehyde, 2-3 butanediol, and dibutyl phthalate. Peinado et al. (2016) reported the cooked seafood flavor developed by slurries of the fish powder hydrolysates (FPHs) with a glucose solution (0.05 mL, 80 mmol/mL) and fish oil (1.5 g/100 g) heated at 110° C for 30 min that characterized as 4-heptenal, 2,4-heptadienal, and some pyrazines, 1-octen-3-ol or 1-hepten-4-ol.

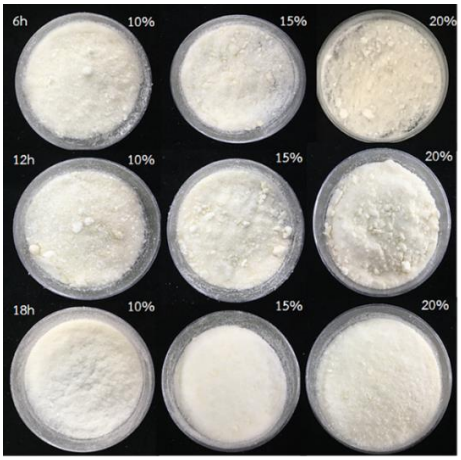

Figure 2. Freeze-dried eb-JPHs obtained from different bromelain concentrations (10, 15, and 20% w/w) and hydrolysis time (6, 12, and 18 h)

Table 2. Volatiles flavor obtained by the reaction of eb-JPH, sugars, and arginine

Flavor compound	RI (Retention index)	Relative peak area (%)		Odor description*
		JGR[1]	JGRA[2]	
Hexanal	1089	3.44	2.87	grass, fishy, tallow, fat
2-Butanone	906	3.80	3.63	sharp, sweet
3-methyl- butanal	922	5.31	4.74	nutty, almond
2-Butanol	1033	5.02	3.32	-
1-hydroxy-2-propanone	1308	3.41	3.36	sweet
Furfural	1474	6.82	5.60	cooked rice, nutty, roasted, sweet
Nonanal	1403	3.55	3.47	sweet. citrusy
Benzaldehyde	1537	3.57	3.64	nutty
Propanoic acid	1561	9.50	2.72	
3-methyl- butanoic acid	1697	4.58	-	sharp, sweet, green, apple
1-methyl-2-pyrrolidinone	1700	4.96	3.76	-
Pyrazine	1220	-	3.59	nutty
2,6-dimethyl- pyrazine	1276	-	3.56	nutty, cocoa, roasted meat
2-Furanmethanol	1670	-	2.88	caramel, coffee, sweet

Note: [1]JGR refers to the process from a reaction mixture of eb-JPH and sugars (glucose and ribose)
[2]JGRA refers to the process from reaction mixture of eb-JPH, sugars (glucose and ribose), and arginine
*odor description (Shimoda and Shibamoto,1990)

4. CONCLUSION

The reduced size of jellyfish by-product powder affected the degree of hydrolysis and yield of jellyfish protein hydrolysate. Bromelain-assisted hydrolysis showed the highest DH and yield of eb-JPH when the condition of 20% bromelain concentration hydrolyzed for 18 h at pH 6 and 50°C was applied. The reaction of eb-JPH, sugars (glucose and ribose), and arginine incubated at 95°C for 2 h generated a meat-like flavor.

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