Identification of Volatile Compounds in Jellyfish Protein Hydrolysate

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Abstract

In the present investigation, jellyfish protein hydrolysates (JPHs) of the umbrella or oral arm of the sand jellyfish (Rhopilema hispidum) and white jellyfish (Lobonema smithii) were produced by acetic acid hydrolysis with the aid of temperature and pressure. Volatile flavor compounds found in the hydrolysates were categorized into 6 groups: aldehydes (hexanal, heptanal, octanal, and 2-butyl-2-octenal), furan (2-butylfuran), terpene (beta-terpinol), alkane (2, 4-dimethyl undecane), acid (pterin-6-carboxylic acid), ester (isobornyl formate) and ketones (β -ionone and propanone). Hexanal, heptanal and octanal that are indicators of fishy flavors were accentuated by acetic acid treatment.

Keywords: Jellyfish protein hydrolysate, Fishy flavor, Off flavor, Volatile flavor compound

1 Introduction

White jellyfish (Lobonema smithii) and sand jellyfish (Rhopilema hispidum) are the most important edible jellyfish species caught in Thailand. Apart from these two species, red type or China type (Rhopilema esculentum), river type (Acromitus flagellatus), prigi type (Crambione mastigophora), ball type (Crambionella orsi), cilacap type (Crambionella sp.) and one unidentified species (semi-China type) are commercially sold in the Southeast Asian market [1]. Salted jellyfish from Thailand are mainly exported to Japan, South Korea, Malaysia, China and Taiwan with a total value close to 10 million US dollar annually [2]. The typical salted jellyfish produced by salting with common salt (sodium chloride), alum (potassium aluminium sulfate) and soda (sodium bicarbonate) [3-4] generally have a marine, iodized or salty, fishy and slightly oxidized flavor. The major protein of processed jellyfish has been identified as collagen [1,3-6], but the production of jellyfish protein hydrolysate (JPH) has not been extensively studied due to the low availability of commercial salted jellyfish in the market.

Fisheries hydrolysate products generally have a fishy flavor, thus limiting their use in foods. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) are generally accepted as indicators of the lack of freshness for seafood products. The volatile compounds contributing to fishy flavor include alkanals (C5-C10), 2-alkenals (C5-C10), trans, trans-2, 4-heptadienal, 2 alkanones (C3-C11), 1-octen-3-one, cis, cis, trans-2, 4, 7-decatrienal and 1-penten-3-one [7]. Fishy flavor compounds of sardine (Sardinops *melanostica*) including hexanal, cis-4-heptenal, trans, cis-2, 6-nonadienal, 1-penten-3-one, 2, 3-pentanedione, methional and TMA have been identified [8]. Trimethylamine is a key compound of marine and fishy odors in sea fig (Microcosmus sulcatus) [9]. The key fishy compound in cooked American lobster tail meat was cis-4-heptenal while many other fishy volatile compounds remain unidentified [10]. On the other hand, dimethyl disulfide is recognized as the

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principal fishy volatile flavor compound from mussel (Mytilus edulis) [11]. Therefore, volatile compounds contributing to fishy flavor may vary from one product to another. The oxidation of polyunsaturated fatty acids (PUFAs) including oleic, linoleic, linolenic and arachidonic generates many volatile aldehydes, and causes rancid, oxidized or off-flavors [12] that reduce consumer acceptance. Acetic acid is a mild food acidulant with a potent vinegar flavor that has various applications in food products. Acetic acid, gluconic acid and trisodium acetate have been used to reduce or mask the fishy flavor or off flavors of processed sea food [13]. The aim of this study was to investigate volatile flavor compounds of JPHs prepared by acetic acid hydrolysis of the umbrella or oral arm portions of the white jellyfish (Lobonema smithii) and sand jellyfish (Rhopilema hispidum).

2 Materials and Methods

2.1 Raw material

The umbrella portion and oral arms of salted white jellyfish (*Lobonema smithii*) and sand jellyfish (*Rhopilema hispidum*) were purchased from Mahachai Seafood, Co., Ltd. Samutsakorn, Thailand. To maintain the quality of the salted jellyfish, the samples were stored at $10\pm2^{\circ}$ C for 3 months in the sealed polyethylene bag.

2.2 Chemicals

Food grade acetic acid (5%) was purchased from a local convenience store. Standards of nineteen amino acids with the purity > 99% were purchased from Sigma-Aldrich Ltd. (St. Louis, MO. USA). Thirty seven fatty acids (Food Industry FAME Mix, Restek) were purchased from C.E. Combination Co., Ltd, Nonthaburi, Thailand. All other chemicals used in this study were of analytical grade.

2.3 Preparation of JPH

Salted jellyfish were washed with tap water 4 times to remove salt and impurities before being immersed in tap water overnight. The desalted jellyfish was drained for 30 min and then chopped into small pieces. The mince (70 g) was blended with 100 ml of 0.01% acetic acid using a Moulinex blender. The same amount of mince mixed with distilled water was used as a control. The slurry was then autoclaved at 121°C, 15 lb/in² for 15 min. The homogenates were concentrated by a rotary evaporator (Rotavapor[®] R-210 Buchi Labortechnik AG, Glatthalde, Flawil, Switzerland) until the soluble solids were approximately 10°Bx. The supernatant was filtered through filter paper to remove the non-hydrolyzed portion and kept frozen until analysis. In this study, the hydrolysates of the umbrella and oral arm of white jellyfish and sand jellyfish are referred to as UW-JPH, OW-JPH, US-JPH and OS-JPH, respectively.

2.4 Analyses

2.4.1 Moisture

Moisture of desalted jellyfish was determined according to AOAC method, 934.01 [14].

2.4.2 Determination of total volatile bases nitrogen (TVB-N) and trimethylamine (TMA)

TVB-N and TMA of desalted jellyfish were also measured. TVB-N was followed the method of Vyncke, et al. [15] and expressed as milligrams per 100 g of desalted jellyfish (wet weight). TMA was performed according to AOAC method, 971.14) [14].

2.4.3 Determination of fatty acid profile

Crude fat content was extracted with petroleum ether (AOAC method, 948.15) [14]. Fatty acid profiles of desalted jellyfish were analyzed. Fatty acid methyl ester was prepared using the boron trifluoride method Thirty seven fatty acids (Food Industry FAME Mix, Restek, C.E. Combination Co., Ltd, Nonthaburi, Thailand) were used as standards. One µl of esterified JPH sample was injected onto a gas chromatograph (GC-6890, Agilent Technologies, Inc. Palo Alto, CA, USA) equipped with a polar biscyanopropyl column (100 m \times 0.25 mm i.d \times 0.2 μ m film, Supelco SPTM 2560, Sigma-Aldrich, St. Louis, MO. USA.) coupled to a flame ionization detector. The initial temperature of column was set to 100°C and held for 5 min. The final temperature was set at 230°C with an increased rate of 4°C per min. The flow rate of the helium carrier gas was maintained at a constant rate of 0.8 ml/min.

The peaks of fatty acids were quantified and expressed as a percentage of the total fatty acids.

2.4.4 pH measurement

The pH measurements of JPHs in acetic acid solution (20 ml) were performed using a pH meter (Cyberscan Model 500, Euteon Instrument, Singapore).

2.4.5 Amino acid analysis

Hydrolysate samples (50 mg) of UW-JPH, OW-JPH, US-JPH and OS-JPH were hydrolyzed in 5 ml of 6 N HCl at 110°C for 24 hr in a block heater (Model SBH 130D, Stuart Scientific, Manchester, UK). After the samples were concentrated by flushing with nitrogen gas, they were adjusted to 5 ml with distilled water and filtered through a 0.45 µm cellulose acetate filter (VertiPure[™] CA Syringe Filter, Vertical Chromatography Co., Ltd. Bangkok, Thailand). Amino acid analysis was performed according to the method of Yan et al., [16]. Briefly, a 10 µl aliquot of each sample was analyzed by reverse phase-high performance liquid chromatography (RP-HPLC Model 1200, Agilent Technologies, Inc. Santa Clara, CA, USA) on a fused silica capillary (C18; 250 mm \times 4.6 i.d., 5µm film thickness; Prevail[™] column Alltech[®], Deerfield, IL, USA). Mobile phase used was 5 mM heptafluorobutyric acid (HFBA) in 0.5% trifluoroacetic acid (TFA) (A) and acetonitrile: H₂O (95:5) (B). Gradient conditions of B were 0, 0, 15, 35% at 0, 3, 8 and 17 min, respectively. Flow rate used was 1.0 ml/min.

2.4.6 Identification of volatile compounds in JPHs by headspace GC-MS

Hydrolysates (5ml) were packed in a 10 ml brown vial for the headspace technique. The samples were heated at 85°C for 30 min in a GC-MS heating block. Using a 2.5 ml gastight syringe with a 23 gauge part no.5 needle (Model 1002 LTN CTC, Hamilton Bonaduz AG., Bonaduz, GR, Switzerland), a 1 ml sample was injected into a gas chromatograph-mass spectrometer (HP 5890, Agilent Technologies, Inc. Palo Alto, CA, USA). Volatile compounds were separated using HP-3MS capillary column (30 m length \times 0.25 mm i.d.; coated film thickness: 0.25 µm). The operating

temperature program was initially maintained at 40°C for 2 min, then increased to 250°C at 4°C per min, and finally held at 250°C for 10 min. The injection temperature was set at 220°C on the splitless mode. Helium gas (99.99%) was used a carrier at a flow rate of 1.5 ml/min. The mass spectrometer (5975 C inert XL EI/CI MSD with a triple-axis detector, Agilent Technologies, Inc. Santa Clara, CA, USA) was operated in scan mode from m/z 40 to 450, with 70 eV at 230°C. The volatile flavor compounds were identified by first comparing their mass spectrum with those in Wiley 275 and the NIST library at percentage of quality match over 85% and compared with previously published literature, followed by retention index (RI values) of JPHs, which was calculated against the standard alkanes C11-C20 using the equation proposed by Van den Dool and Kratzs [17] as indicated below.

RI = 100 ([Rt(x)-Rt(z)] / [Rt(z+1)-Rt(z)]) + 100Z

Where	Rt(x):	retention time (min) of the interested compound
	Rt(z):	retention time (min) of normal alkane
	Rt(z+1):	retention time (min) of z+1 normal alkane

Z: the number of carbon atoms

2.5 Statistical analysis

The analyses of moisture, TVB-N, TMA and pH were performed in triplicate while the determinations of amino acid, fatty acid profile, and volatile compounds were carried out in duplicate. The data were analyzed by SPSS and Duncan's multiple range test was used to determine the significant differences (p < 0.05) among means [18].

3 Results and Discussion

3.1 Chemical quality of desalted jellyfish

All desalted jellyfish samples showed no significant difference in moisture. The moisture content of white and sand jellyfish was approximately 95%. The analysis of fatty acid profile of desalted samples was performed due to the fact that polyunsaturated fatty

acid may produce oxidized flavor compound during hydrolysis. No short chain fatty acids were found in all desalted samples. These two types of desalted jellyfish had medium and long chain fatty acids (Table 1). The ω -6 fatty acids found in both desalted jellyfish were linoleic acid and arachidonic acid. Additionally, the ω -3 fatty acids including cis-9, 12, 15-octadecatrienoic acid and cis-5, 8, 11, 14, 17-eicosapentaenoic acid were found only in the umbrella and oral arm portions of sand jellyfish. Although the amount of polyunsaturated fatty acid in desalted jellyfish was low, oxidation of these fatty acids could occur during storage [19].

TVB-N and TMA compounds are commonly used as indicators of the (lack of) freshness of seafood.

The TVB-N contents of umbrella and oral arm of desalted white jellyfish and sand jellyfish were 12.8 ± 0.20 , 17.5 ± 0.29 , 12.8 ± 0.40 and 15.2 ± 0.53 mg/100g, while that of TMA was 8.2 ± 0.20 , 7.0 ± 0.00 , 7.0 ± 0.00 and 9.3 ± 0.20 mg/100g, respectively. The TVB-N level was below the range of 30-35 mg/100g that is the acceptability limit for ice stored cold-water fish [20-21]. The high content of both TVB-N and TMA suggests that, in general, the jellyfish might already be at an initial stage of decomposition prior to salting; hence, the typical intense fishy flavor could detect from salted jellyfish samples. In this study, the washing step with water several times can reduce the fishy and salty smell of the salted samples.

Table 1	Profile	of fatty	acid of	desalted	jellyfish	samples*
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		% Fatty acid					
Type of fatty acid	RT (min)	white j (<i>Lobonen</i>	ellyfish na smithii)	sand jellyfish (<i>Rhopilema hispidum</i>)			
		umbrella	oral arm	umbrella ³	oral arm ⁴		
Saturated							
dodecanoic acid	23.53	0.52	0.87	< 0.00	0.43		
tetradecanoic acid	27.54	7.92	4.67	2.77	3.28		
pentadecanoic acid	29.40	2.23	1.87	1.19	1.29		
hexadecanoic acid	31.16	43.64	40.81	39.76	40.68		
heptadecanoic acid	32.83	2.71	3.79	3.24	3.27		
octadecanoic acid	34.43	17.64	23.06	21.31	22.68		
eicosanoic acid	37.42	1.08	1.85	0.98	1.46		
heneicosanoic acid	38.85	0.71	0.55	0.36	0.37		
docosanoic acid	40.32	1.34	1.35	0.84	0.71		
Tricosanoic acid	41.91	< 0.00	< 0.00	0.20	0.17		
Tetracosanoic acid	43.50	1.33	1.16	0.82	0.79		
Total		79.12	79.98	71.47	75.13		
Monounsaturated							
cis-9-tetradecenoic acid	29.09	< 0.00	< 0.00	0.70	< 0.00		
cis-10-pentadecenoic acid	30.89	< 0.00	< 0.00	< 0.00	< 0.00		
cis-9-hexadecenoic acid	32.37	6.86	2.83	3.69	2.69		
cis-10-heptadecenoic acid	34.01	< 0.00	< 0.00	< 0.00	0.11		
cis-9-octadecenoic acid	35.43	4.18	6.27	7.13	5.38		
cis-11-eicosenoic acid	38.36	< 0.00	< 0.00	< 0.00	1.00		
cis-15-tetracosenoic acid	44.69	0.72	0.74	0.42	0.40		
Total		11.76	9.84	11.94	9.58		
Polyunsaturated							
cis-9,12-octadecadienoic acid	36.91	0.51	1.08	1.46	0.61		
cis-9,12,15-octadecatrienoic acid	38.59	< 0.00	< 0.00	< 0.00	0.23		
cis-13,16-docosadienoic acid	41.34	< 0.00	< 0.00	0.10	0.53		
cis-11,14,17-eicosatrienoic acid	41.62	< 0.00	< 0.00	0.12	< 0.00		
cis-5,8,11,14-eicosatetraenoic acid	41.85	0.56	0.36	0.22	0.27		
Cis-5,8,11,14,17-eicosapentaenoic acid	44.00	< 0.00	< 0.00	0.36	< 0.00		
Total		1.07	1.44	2.26	1.64		

*The umbrella and oral arm of desalted white jellyfish and those of sand jellyfish had the moisture content of 93.52, 92.59, 95.12 and 93.75%, respectively. The calculation was based on wet weight basis.

3.2 Quality of JPHs

The type of amino acid is associated with taste and flavor in food products. Amino acid analysis of all JPHs contained collagen as indicated by their hydroxyproline content (Table 2). Published data for Stomolophus meleagris [5] and Rhopilema esculentum [22] showed that these species have similar amino acid profiles with only minor differences in amino acid content. The amino acid profiles show considerable differences as compared with those of Rhopilema hispidum and Lobonema smithii. In this study, all hydrolysates had almost all of the essential amino acids, except tryptophan. Tryptophan is an acid labile amino acid which may be destroyed during hydrolysate preparation and amino acid determination [23]. Compared with other JPHs, the present results revealed that, in most cases, JPHs had higher contents of the essential amino acids lysine and threonine (Table 2). Non-essential amino acids including glutamic acid,

arginine and tyrosine were also higher in all JPHs (Table 2). JPHs typically give a marine, iodized or salty and oxidized flavor as well as a fishy flavor. When acetic acid was used, it had an additional positive impact owing to the fact that the mild vinegar flavor could partially mask the fishy flavor in the hydrolysate products. However, hydrolysates with an excessively high vinegar flavor would also decrease consumer acceptance. In this study, the condition used for producing JPHs was 0.01% acetic acid and pressure (15 lb/in²) at 121°C. A pH range of all the obtained JPH is between 3.80-3.86. Mild acid hydrolysis under elevated temperature and pressure could destabilize hydrogen bonds and promote hydrophobic and electrostatic interactions, resulting in hydrolysis of the collagen helix [24]. Together with the characteristic vinegar flavor of acetic acid, all JPHs had less 2-butyl-2-octenal and pterin-6-carboxylic acid. The results of this investigation suggested that acetic acid could suppress some flavors of JPHs.

	Sample								
Type of amino acid	US-JPH* (residue/ 1000 residue)	OS-JPH* (residue/ 1000 residue)	UW-JPH* (residue/ 1000 residue)	OW-JPH* (residue/ 1000 residue)	pepsin-solubilised jellyfish collagen ¹ (residue/ 1000 residue)	jellyfish hydrolysate ² (mg/100g)			
Essential									
histidine	7	14	8	7	2	4.4			
isoleucine	20	21	19	18	22	27.7			
leucine	30	33	33	31	34	35.5			
lysine	161	70	97	87	38	26.9			
methionine	10	16	12	11	4	14.3			
phenylalanine	13	15	12	11	10	16.8			
threonine	53	53	51	52	35	28.9			
valine	26	28	26	25	35	55.6			
Non-essential									
alanine	56	60	55	57	82	84.8			
arginine	67	77	73	74	52	50.6			
aspartic acid	78	77	81	83	79	85.4			
glutamic acid	114	111	113	116	98	90.8			
glycine	209	233	244	252	309	289.5			
hydroxyproline	46	67	62	61	40	45.2			
proline	62	73	66	73	82	85.4			
serine	34	38	35	32	45	45.5			
tyrosine	12	17	14	13	6	12.4			
Sum	1000	1000	1000	1000	1000	999.7			

 Table 2: Amino acid profile of JPHs

Remark.

US-JPH referred to hydrolysate from umbrella of sand jellyfish

OS-JPH referred to hydrolysate from oral arm of sand jellyfis

UW-JPH referred to hydrolysate from umbrella of white jellyfish

OW-JPH referred to hydrolysate from oral arm of white jellyfish

¹ results of *Stomolophus meleagris* [5]

² results of *Rhopilema esculentum* [22]

*The data obtained was based on wet weight basis

However, the fishy flavor is quite different from that obtained from spoiled fish. In this study, the contents of a number of volatile flavor compounds of white jellyfish (UW-JPH and OW-JPH) were higher compared to those of sand jellyfish (US-JPH and OS-JPH) (Tables 3 and 4). A total of 9 identified volatile compounds and 2 unknown were discovered in UW-JPH and OW-JPH, while 8 identified compounds were found in US-JPH and OS-JPH. The results revealed that different species and portions of salted

jellyfish had slightly different flavor characteristics. Moreover, gas chromatography results indicated that most of the acid hydrolyzed JPHs had increased relative peak areas as compared with the water hydrolyzed JPHs which were used as control samples in this study. The acid hydrolysis reduced the levels of 2, 4-dimethyl undecane and of two unknown compounds of UW-JPH and OW-JPH and the levels of 2-butylfuran, propanone and pterin-6-carboxylic acid of US-JPH and OS-JPH (Tables 3 and 4).

Table 3: Flavor compounds found in protein hydrolysates of white jellyfish

Eld	RI		- J J**			
Flavor compound	(Retention index)	control	UW-JPH	control	OW-JPH	odor description*
hexanal	810	7.19	31.15	6.69	31.69	grass, fishy, tallow, fat
heptanal	851	2.31	3.04	3.46	8.02	fat, citrus, rancid
unknown	886	2.67	-	4.18	-	-
2-butylfuran	894	-	7.43	-	7.42	non-characteristic, weak spicy
unknown	917	17.34	-	15.34	-	-
octanal	1000	-	5.71	-	5.88	fat, green
2-butyl-2-octenal	1126	7.66	12.54	6.53	18.22	meat
2,4-dimethyl						
undecane	1198	5.93	-	4.53	-	-
β-ionone	1423	3.55	18.67	3.51	11.26	floral, woody, sweet
propanone	1435	3.91	9.47	3.75	6.54	-
pterin-6-carboxylic acid	1968	49.47	11.94	50.11	10.42	-

Remark

UW-JPH referred to the hydrolysate from umbrella of white jellyfish

OW-JPH referred to the hydrolysate from oral arm of white jellyfish

*odor description [32]

 Table 4: Flavor compound found in protein hydrolysates of sand jellyfish

F1	RI		1 1 • 4• *			
Flavor compound	(Retention index)	control	US-JPH	control	OS-JPH	odor description*
hexanal	810	30.60	31.69	41.73	38.78	grass, fishy, tallow, fat
heptanal	851	7.04	7.99	7.80	11.15	fat, citrus, rancid
2-butylfuran	894	11.40	8.42	10.50	8.16	non-characteristic, weak spicy
octanal	1000	-	9.27	-	7.14	fat, green
2-butyl-2-octenal	1126	11.75	22.02	6.05	2.60	meat
2,4-dimethyl undecane	1198	7.24	-	4.53	8.37	-
propanone	1435	6.48	4.54	6.51	-	-
pterin-6-carboxylic acid	1968	25.46	10.62	22.84	20.00	-

Remark

US-JPH referred to the hydrolysate from umbrella of sand jellyfish OS-JPH referred to the hydrolysate from oral arm of sand jellyfish *odor description [32].

The alkane, 2, 4-dimethyl undecane, was also reduced in US-JPH but increased in OS-JPH. All eight volatile flavor compounds were identical in sand jellyfish (US-JPH and OS-JPH) and white jellyfish (UW-JPH and OW-JPH), except that white jellyfish hydrolysates had additionally β -ionone and two unknown compounds (Tables 3 and 4). Identified volatile flavor compounds (tables 3 and 4). Identifie

Volatile aldehydes including hexanal, heptanal and octanal detected in all JPHs demonstrated fishy, oily or fatty flavors. However, no TVB-N or TMA was detected since pH of the hydrolysate was acidic pH (3.8), so that volatile base was ionic and was not well volatilized. Octanal, which has a fatty pungent flavor, was found mostly in hydrolysates of sand jellyfish. Fishy, fatty, rancid or unpleasant odors in these JPHs could have been derived from lipid oxidation [8,12,19,25], as in the case with other processed marine products during storage. 2-Butylfuran was found in all JPHs. A furan could have been derived from Maillard reactions that occurred during the heating and sample concentration step by rotary evaporation [12,26]. Small quantities of β -ionone and propanone found in the hydrolysates could have originated from the degradation products of algae that are symbiotic with jellyfish. β -ionone that contributes to floral and woody flavors was evident particularly in UW-JPH. The ketone could be attributed to the beta-carotene compounds constituting in foods that jellyfish consumed [27], which were afterwards oxidized during storage. These compounds would have accumulated in the jellyfish and oxidized during storage. Pterin-6-carboxylic acid is the end product of folic acid degradation that yields p-aminobenzoyl-L-glutamic acid and 6-formyl pterin. Exposed to ultraviolet radiation, 6-formyl pterin is transformed to pterin-6-carboxylic acid [28]. 2-butyl-2-octenal has identified in fresh lamp [29] and cured pork [30] and so its presence in jellyfish is not surprising. Due to the fact that jellyfish comprises mainly water, they easily absorb and accumulate water-soluble contaminating compounds that potentially add to off flavors in the product. Guttman and Van Rijn

reported that geosmin and 2-methyl isoborneol produced by cyanobacteria, fungi or actinomyces caused earthy-musty or off flavor in fish such as tilapia [31]. From the results of this study, GC-olfactometry (GC-O) may have to be deployed to further characterize the JPHs.

4 Conclusions

Jellyfish can be hydrolyzed by mild acetic acid with the aid of heat and pressure to produce protein hydrolysates. The hydrolysates of the two types of jellyfish, white jellyfish and sand jellyfish, showed slight differences in amino acid content and odor. Hydrolysates of jellyfish protein had marine, salty or iodized, oxidized and fishy flavor. The compounds hexanal, heptanal, 2-butylfuran, octanal, 2-butyl-2-octenal, 2, 4 dimethyl undecane, β -ionone, propanone and pterin-6-carboxylic acid were detected in JPHs. Acetic acid may enhance the aldehyde volatiles (hexanal, heptanal and octanal), but it may mask the flavors from 2-butyl-2-octenal and pterin-6-carboxylic acid of JPHs. JPHs with low fishy flavor may be used as a functional and/or seasoning ingredient in food products.

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