

INTRODUCTION

Milkfish (*Chanos chanos*) in Indonesia is an economically valuable fish which become a popular aquaculture commodity because its savory taste and the price is affordable. Total production of milkfish in 2014 reached 631.125 tons, or 14.74% of the total production of farmed fish. The increase in milkfish production from 2010 to 2014 reach an average of 10.84%. Meanwhile, milkfish production in 2015 will increase by 865.93 tons (KKP 2016).

Dishes made from raw fish can be processed using heat, whether it is by boiling or steaming. Basic properties of the fish meat which were used as raw material will be influenced by steaming process which could caused several changes in flavor and texture when the raw materials are going through processing steps (Sartika 2009). Steaming or the use of steam as a heat source has the advantage of minimalizing the risk of vitamins and other dietary components loss which usually are sensitive to heat (Fellows 2000).

Flavor is a sensation which is produced when meat or food is placed in the mouth mainly incurred by the taste and aroma. Volatile components is a component that gives aroma sensation. The composition of volatile flavor compounds which were detected in fishery products usually derived from the class of aldehydes, alcohols, ketones, acids and hydrocarbons (Pratama et al., 2013).

Studies and research information regarding the composition of volatile flavor compounds in Indonesia's fishery commodities were not easily available while different things found abroad, volatile flavor of processed fishery products research have been carried out since many years back, as example research which were carried out by Liu et al. (2009) regarding the effect of cooking and re-cooking on the volatile and

non-volatile compounds found in silver carp (*Hypophthalmichthys molitrix*).

Flavor components research is essential considering the identification of a certain components up until a complete composition fo flavor compounds or group of compound in a fishery commodity would help to support the documentation process of distinctive fishery products based on their flavpr composition thus protecting its authenticity. The purpose of this study is to identify the volatile flavor compounds contained in fresh and steamed milkfish (*Chanos chanos*)

MATERIALS AND METHODS

Material and Equipment

The samples were taken from Karangsong fish landing site, Indramayu District, West Java, Indonesia. The research was conducted between February and April 2018, at the Laboratory of Fishery Processing Products (sample preparation), Faculty of Fisheries and Marine Sciences (FPIK), Padjadjaran University (UNPAD), proximate analysis conducted at the Laboratory of Inter University Centre and volatile compounds analysis conducted at Flavor Laboratory, Rice Research Centre Office, Sukamandi, Subang.

Chemicals for proximate analysis, namely HCL, CuSO₄, K₂SO₄, NaOH, H₂SO₄, and chloroform). The equipment used in this research including steamer, Kjeldahl flask, cling wrap, aluminum foil, electric scales (Tanita), furnaces, stoves, ovens, soxhlet apparatus, knives, zip-lock plastic, paper labels, waterbath and gas Chromatography (Tecnologies Agilent 7890A GC System) / Mass Spectrometry Technology Agilent 5975C inert XL EI CI / MSD.

This study use experimental method with fresh and steamed milkfish as treatments. Both of these treatments are identified their volatile flavor compounds

and their proximate content (moisture, ash, protein and fat). Treatment for proximate analysis was carried out in triplicate and then the both results are analyzed descriptively.

PROCEDURES

Sample's Preparation

Milkfish was taken as much as 5 kg from Karangsong, Indramayu, West Java and transported in a coolbox to UNPAD Fisheries Processing Product Laboratory to be prepared before analysis. Furthermore, at the laboratory the fish were gutted, cleaned and then divided into two groups, fresh and steamed. The steamed group were steamed for 30 minutes at a temperature of 100°C. Both groups were then packed in a triple layered packaging (aluminum foil, cling wrap and zip-lock plastic). This was done to minimize changes and damage to samples flavor which can be caused by air, light, and temperature (Pratama 2011). Samples which have been tightly packaged were then inserted into the low-temperature coolbox to be transported to the respective laboratory analysis.

Proximate analysis

Proximate analysis carried out on samples of fresh fish and steamed consists of the analysis of water content, ash content, protein and lipid based on AOAC (2005) procedures. The data obtained from all samples were calculated their average and standard deviation value and then was discussed descriptively.

Analysis of Volatile Compounds

Analysis volatile compounds was performed using a series of Gas Chromatography (GC) and Mass

Spectrometry (MS) apparatus. Extraction of samples was carried out with Solid Phase Micro Extraction (SPME) method using fiber DVB/ Carboxen/Polydimethylsiloxane. The temperature used for sample extraction was 35°C for fresh sample and 80°C for steamed sample for 45 minutes (in water bath). GC column used was HP-INNOWax (30 m x 250 µm x 0.25 m), helium gas, initial temperature used was 45°C (hold 2 min), with increase in temperature of 6°C/minute, and final temperature of 250°C (hold 5 minutes) with total time of 32.775 minutes. Mass spectra of compounds detected were then compared to mass spectra patterns in the data center or library version 0.5a NIST (National Institute of Standards and Technology) on a computer database. The components of the volatile flavor compound were further analyzed using the Automatic Mass Spectral Deconvolution and Identification System (AMDIS) software (Mallard and Reed 1997).

RESULTS AND DISCUSSION

Analysis of Volatile Compounds

The analysis result of fresh milkfish sample's volatile compounds successfully detected 11 volatile compounds (Table 1), while the analysis result of steamed sample's volatile compounds successfully detected 52 volatile compounds (Table 2). . Compounds in steamed fish samples were higher than fresh fish. The volatile flavor compounds identified were categorized into several groups such as hydrocarbons, aldehydes, alcohols, ketones, esters and furans.

Table 1. The volatile compounds of fresh milkfish

No.	Group	Retention Time	Compound	Area	Proportion (%)
1	Hydrocarbons	24.5278	<i>Pentadecane</i>	4447761	76.16
2		28.176	<i>Hexadecane</i>	945485	16.20
3		26.3885	<i>Tetradecane</i>	103200	1.77
4		25.7067	<i>Undecane</i>	10276	0.18
5		18.3641	<i>Azulene</i>	703	0.01
6	Aldehyde	11.7997	<i>Benzaldehyde, 4-ethyl-</i>	279012	4.78
7		22.5136	<i>2-Pental</i>	2859	0.05
8		6.1425	<i>Pental</i>	2235	0.04
9	Alcohol	16.1439	<i>1-Pentanol</i>	40634	0.67
10		25.7191	<i>1-Heptanol</i>	2781	0.05
11	Ketones	20.4551	<i>2,3-Pentanedione</i>	4939	0.08
Total Area				5839885	100

Table 2. Volatile compounds of steamed milkfish

No.	Group	Retention Time	Compound	Area	Proportion (%)
1	Hydrocarbons	24.6022	<i>Pentadecane</i>	74164023	64.89
2		28.2245	<i>Hexadecane</i>	22133625	19.37
3		28.0817	<i>1-nonadecene</i>	3264251	2.86
4		22.5414	<i>Tetradecane</i>	1184586	1.04
5		27.9913	<i>8-Heptadecene</i>	537569	0.47
6		25.7197	<i>1-Iodo-2-methylundecane</i>	172819	0.15
7		31.4955	<i>Nonadecane</i>	131679	0.12
8		24.1908	<i>1-Pentadecene</i>	128383	0.11
10		24.8402	<i>Decane</i>	122245	0.11
11		18.3111	<i>1,3,6-Heptatriene, 5-methyl-</i>	45798	0.04
12		14.4161	<i>D-Limonene</i>	20799	0.02
13		14.6156	<i>Cyclopentene</i>	340	0.00
14		Aldehyde	16.1422	<i>Nonanal</i>	2908511
15	28.5037		<i>2-Undecenal</i>	840 487	0.74
16	30.2434		<i>Hexadecanal</i>	787 727	0.69
17	13.7106		<i>Octanal</i>	645 496	0.56
18	24.3897		<i>2-nonenal, (E) -</i>	406 119	0.36
19	26.7068		<i>Dodecanal</i>	157 308	0.14
20	17.7402		<i>Benzaldehyde, 4-ethyl-</i>	153 795	0.13

No.	Group	Retention Time	Compound	Area	Proportion (%)
21		18.4823	<i>Decanal</i>	142 942	0.13
22		11.2442	<i>Heptanal</i>	120 211	0.11
23		8.8847	<i>Hexanal</i>	56440	0.05
24		19.7751	<i>2-nonenal, (E) -</i>	44633	0.04
25		19.9587	<i>2,4-Heptadienal, (E, E) -</i>	37642	0.03
26		30 422	<i>2,6-Nonadienal, (E, Z) -</i>	30422	0.03
27		21.9464	<i>2-Octenal, (E) -</i>	21994	0.02
28		21.0189	<i>2,4-Hexadienal, (E, E)</i>	18867	0.02
29		22.8135	<i>2-Hexenal, (E) -</i>	9687	0.01
30		13.7307	<i>Pentanal</i>	6298	0.01
31		19 964	<i>2-Pentenal, (E) -</i>	1317	0.00
32		29.7735	<i>2-Heptenal, (E) -</i>	2342	0.00
33	Alcohol	6.9236	<i>Cyclobutanol</i>	2261107	1.98
34		13.2041	<i>1-Octen-3-ol</i>	1752072	1.53
35		15.4025	<i>1-Octanol</i>	144297	0.13
36		25.5934	<i>1-Nonanol</i>	86858	0.08
37		27.9217	<i>1-Heptacosanol</i>	25900	0.02
38		25.8667	<i>1-Hexanol</i>	7994	0.01
39		31.8066	<i>1-Hexanol, 2-ethyl</i>	7558	0.01
40		29.1673	<i>1-Octanol, 2-butyl</i>	1012	0.00
41	Ketones	13.2207	<i>2,3-Octanedione</i>	1434508	1.26
42		15.9757	<i>3,5-Octadien-2-one</i>	45575	0.04
43		20.1476	<i>2-decanone</i>	16482	0.01
44		15.6139	<i>2,3-Pentanedione</i>	9481	0.01
45		18.4947	<i>3-Heptanone, 6-methyl-</i>	2775	0.00
46	Esters	28.9311	<i>Carbonic acid, dodecyl prop 1-en-2-yl ester</i>	15894	0.01
47		29.0669	<i>Sulfurous acid, pentadecyl 2-propyl ester</i>	18608	0.02
48		29.2617	<i>Carbonic acid, prop-1-en-2-yl ester tetradecyl</i>	30681	0.03
49		30.4247	<i>Oxalic acid, pentadecyl cyclobutyl ester</i>	11712	0.01
50	Furan	33.9725	<i>Furan, 2-ethyl-</i>	5585	0.00
51		34.5216	<i>Nonahexacontanoic acid</i>	32995	0.03
52		29.6809	<i>Cyclobutane, 1,1-dimethyl-2-octyl-</i>	77221	0.07
Total Area				114286670	100.00

Most of the compounds detected can be categorized into hydrocarbons, aldehydes, ketones groups. According to Pratama *et al.* (2013), in general it can be concluded that the steamed sample will have more volatile compounds more compared to fresh samples. The process which involves heat such as steaming is one of the factors which affect the identified volatile compounds.

The results of volatile flavor compounds analysis showed that the compounds can be categorized into several major groups. There were 5 compounds in fresh milkfish sample and 13 compounds in the steamed samples which can be categorized into hydrocarbon groups. According to Pratama *et al.* (2017), hydrocarbon volatile compounds derived from decarboxylation reaction and separation processes of fatty acid carbon chain, a secondary reaction of carotenoids (if any) and thermal oxidation of unsaturated fatty acids.

Aldehyde group identified on fresh fish samples successfully detected 3 compounds, while the steamed samples detected 19 compounds. *Benzaldehyde-4 ethyl* compound has the largest proportion in this group (4.78%), whereas in the steamed samples, nonanal compound has the largest proportion (2.54%). According to Pratama *et al.* (2013), aldehyde group derived from oxidation of the unsaturated fatty acids double bonds or saturated fatty acid which contained in fish meat.

The alcohol group detected in fresh samples consist of 2 compounds, whereas 8 alcohol compounds were detected in steamed samples. Cyclobutanol compound has the highest proportion of all compounds

identified in steamed samples (1.98%). According to Pratama *et al.* (2017), cyclobutanol compounds found in shrimp impart certain aroma and contribute to quality of commodities.

Ketone group detected in fresh milkfish sample consist of 1 compound and in steamed sample consist of 5 compounds were identified. Compounds which has the highest proportion of all compounds identified in steamed samples is 2,3-*Octanedione* (1.26%). According to Liu *et al.* (2009), ketone group compounds can be produced from thermal oxidation or degradation of unsaturated fatty acids, degradation of amino acid, or oxidation caused by microorganisms.

There are two more groups which not to common found in steamed and fresh fish and they are furan and ester. Furan and furfural are decomposition products of cellulose and hemicellulose wood. Some furans can also be produced through the Maillard reaction (Chung *et al.* 2002). Furan compounds detected could be derived from environmental pollution. Esters in general are basic elements with high flavor formation properties (Toth & Potthast 1984). The same thing was stated by Guillen and Errecalde (2002), that ester in general, is considered important for food flavor, especially in fruit. Esters found in fish may originate through acid esterification with alcohol previously formed from lipid metabolism (Guillen & Errecalde 2002)

Proximate analysis

Proximate analysis results of fresh and steamed milkfish samples can be seen in (Table 3) as average value from three replicates and their standard deviation.

Table 3. Proximate analysis result of fresh and steamed milkfish (%)

Parameters	Fresh	Steamed
Water content	72.73 ± 0.02	67.13 ± 0.22
Ash	1.90 ± 0.007	1.98 ± 0.02
Lipid	1.98 ± 0.13	3.81 ± 0.07
Protein	20.25 ± 0.53	22.92 ± 0.71

The proximate analysis results showed that there was a difference between fresh and steamed samples water content. The steamed sample had lower water content when compared to fresh milkfish samples. According to Fellows (2000), treatment with high temperature vapour as steaming can eliminate the water content in intercellular spaces between cells and this is what causes the water content in the steamed sample was measured lower compared to fresh one.

Analysis results showed that there was an increasing of ash content in fresh sample compared to steamed sample. The ash content which contained in the raw materials can be affected by the fish type, growth stage, environmental factors and also feed consumed by fish during growth.

Proximate analysis result showed that an increase in lipid content in steamed fish compared to fresh samples. According to Pratama et al., (2013) and Doe (1998), the higher the loss of water content from the samples then the greater lipid content (and the content of other nutrients) measured on the proximate analysis.

The analysis results showed that the protein content of steamed sample were higher compared to fresh sample after they were going through the cooking (steaming) process. Differences in protein levels measured in both treatment may be caused

by external factors such as environment, season and processing methods such as heating. According Sebranek (2009), protein content can be increased as a result of processing involving a high temperature. This could be caused due to the release of water from the fish meat so that the protein was more concentrated. Hence, the water content contained in samples will also greatly affect the protein content measured in fresh and steamed milkfish samples.

Conclusion

Based on this research it can be concluded that the volatile compounds which were detected in fresh milkfish samples are 11 compounds and 52 compounds in steamed milkfish sample. Most of the compounds which were detected can be categorized as hydrocarbons, aldehydes, alcohols and ketones groups. Hydrocarbons is the most identified volatile compounds group (13 compounds) in fresh milkfish samples while aldehydes (19 compounds) is the most identified volatile compounds group in steamed sample.

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