VOLATILE COMPONENTS COMPOSITION OF FRESH SQUID (*Loligo* sp.)

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ABSTRACT

The components of volatile compounds contained in a commodity have an influence on the distinctive aroma and flavor of the commodity. One of the fishery commodity with a fairly high production volume in Indonesia is squid. Squid samples was taken from Indramayu, West Java, Indonesia and subsequently the sample preparation was conducted at the Laboratory of Fishery Processing Technology, Faculty of Fisheries and Marine Science, Universitas Padjajaran. Volatile components were analyzed at Flavor Laboratory, Indonesian Center for Rice Research, Subang. Volatile compounds were analyzed using Gas Chromatography / Mass Spectrometry (GC / MS) with an extraction temperature of 80ºC in 45 minutes (Solid Phase Micro Extraction). The proximate analysis was analyzed at Inter-University Centre Laboratory, Bogor Agricultural Institute. The volatile compound analysis successfully detected 15 compounds in the fresh squid sample. The proximate analysis showed fresh squid contained 84.01% water content, 1.01% ash content, 0.65% fat content and 14.13% protein content.

Keyword: *aroma, flavor, proximate, squid, volatile*

1. INTRODUCTION

Indonesia has the potential for various types of marine resources such as large pelagic fish, small pelagic fish, shellfish, shrimp, lobster, and squid. The entire potential of the captured resources has a fairly high production volume value. Squid is one of the marine products that is quite abundant in Indonesian waters and is quite in demand by the public. Squid export results in 2016 reached 5,579.32 tons per year. Squid production in Indonesia is estimated at 20.74 tons per year [1].

Squid has no spine, is easy to digest, has a distinctive taste and aroma, and contains essential amino acids such as leucine, lysine, and phenylalanine that are needed by the body. In addition, the dominant levels of non-essential amino acids in squid meat are glutamic acid and aspartic acid which contribute greatly to the emergence of savory and savory effects [2]. Organic elements found in squid meat can produce a distinctive aroma. According to [3] squid meat is also rich in minerals such as phosphorus and calcium which are useful for growth and bone development.

The basic characteristics of each food ingredient as a raw material can change due to various factors and generally differ between each ingredient. The chemical composition of each fresh food ingredient and material that has gone through the processing process is of course also different, possibly due to a series of chemical reactions resulting in a different composition of flavor compounds. In other words, the type of food ingredients can affect the composition of product flavor compounds in general.

Flavor is a sensation that occurs when eating or drinking caused by volatile and nonvolatile chemical compounds, and can be natural or synthetic. In other words, flavor is a combination of taste and aroma that is felt by the human sensory organs when consuming a food ingredient. The surface of the tongue and oral cavity also reacts to tactile and temperature stimuli, such as the sensation of cold in menthol and hot in pepper. In addition, the tactile senses also respond to texture, astringency, and all that contribute to the overall perception of flavor [4]. Flavor has an important role in organoleptic characteristics [5], including in fishery products. Studying the volatile flavor

components contained in fresh products is one of the important factors in determining the quality of a food ingredient and the quality of product storage [6]. The level of consumer acceptance and consumption preferences of a product can be influenced by flavor because each fisheries commodity has a different characteristics and chemical content.

Volatile components are components that give the impact on aroma and they are evaporate easily. The amount of volatile components released by a product is affected by temperature and its natural components. To obtain volatile components from a food ingredient, an extraction method is needed that can separate the volatile components to be identified from other non-volatile components contained in food's macro and micro molecules, such as protein, carbohydrates, water, fat, minerals and vitamins. [7] examined the volatile components of silver carp using the Solid Phase Microextraction (SPME) analysis method and identified it by means of Gas Chromatography-Mass Spectrometry (GC/MS). Many researches on volatile flavors for fishery commodities have been carried out, but this is not the case with fishery commodities in Indonesia because this type of basic study is still not widely available, hence the data are extremely scarce. This study aims to identify the volatile compounds composition of fresh squid which originate from Indonesian waters.

2. MATERIALS AND METHODS

2.1 Sample's Preparation

Squid samples (2.5 kg) were taken from fish sellers around the Karangsong fish landing site area at Indramayu, West Java Indonesia. Afterward, squid samples were transported using a cool box and slurry ice with a ratio of 1:3 to the Fishery Products Processing Laboratory, Padjadjaran University. Sample preparation was carried out based on the modification of [8]. Fresh squid samples were cleaned, eviscerated and subsequently the meat parts was weighed for identification of volatile compounds and proximate analysis.

Fresh samples that have been weighed, then packed using aluminum foil, labeled and covered with cling wrap and finally placed in zip-lock plastic. The packaged samples were put into a cool box containing crushed ice, then transported to the Flavor Laboratory of Indonesian Rice Research Center, Subang to be analyzed and identified for their volatile flavor compounds and also to the Laboratory for Conservation of Endangered Animals, Inter-University Centre, Bogor Agricultural Institute to be analyzed its proximate content.

2.2 Volatile Compounds Identification

Identification of volatile flavor compounds was carried out based on the modification of [9]. Identification of volatile flavor compounds was completed by means of Gas Chromatography (Agilent Technologies 7890A GC System) and Mass Spectrometry (Agilent Technologies 5975C Inert XL EI CI / MSD) analytical equipment. Samples extraction were performed using the Solid Phase Micro-Extraction (SPME) method to evaporate volatile compounds constituted in the sample. The fiber used was DVB/Carboxen/Poly Dimethyl-Siloxane which function as an absorbent for sample's volatile compounds. A total of 1.5 g of the refined sample was placed into a special 22 ml SPME vial. The extraction temperature of fresh squid samples was 40^{o}C with a heating time of 45 minutes in a water bath (Memmert®). Fiber was then inserted into the sample injector at the top parts of the GC/MS apparatus, afterwards the GC was set for its analysis parameters. The GC column used was HP-INNOWax (30 m x 250 μm x 0.25 μm), with helium carrier gas, and the main running GC parameters used were: initial temperature 45^{o}C (hold 2 minutes), temperature increase at 6^{o}C/minute, final temperature 250^{o}C (hold 5 minutes) with a total running time of 32,775 minutes. The results obtained in the form of a chromatogram and a list of detected compounds were then compared with the mass spectra patterns which built in the computer's data center from NIST (National Institute of Standards and Technology) library version 0.5a.

2.3 Proximate Analysis

Proximate analysis conducted in this study consisted of moisture content analysis (thermo-gravimetric method), ash content (dry ashing), protein content (crude protein) and lipid content analysis (Soxhlet extraction). All analysis procedures were performed according to [10]. Each analysis were carried out in three replications.

2.4 Data Analysis

The detected volatile flavor compound data were further analyzed using Automatic Mass Spectral Deconvolution and Identification System (AMDIS) software [11]. AMDIS automatically extracts pure component mass spectra from highly complex GC-MS data files and uses these purified spectra for a search in a mass spectral library (NIST). The data obtained from the identification of volatile compounds were discussed in a comparative descriptive manner based on the related scientific references and intensity of the semi-quantification compounds that were detected in the fresh squid samples [12]. The average value from three replications of proximate analysis resulting data were determined and expressed with their standard deviation [13]. All the proximate data were then discussed descriptively.

3. RESULT AND DISCUSSION

3.1 Volatile Compound Analysis

The results of the mass spectra analysis of the compounds in the fresh squid sample showed that the volatile flavor compounds identified were originate from the hydrocarbon group of 6 compounds, aldehydes as many as 2 compounds, alcohol as many as 3 compounds, ketones as many as 1 compound, and other groups of compounds as many as 3 compounds. The total number of compounds detected in fresh squid samples was 15 compounds as shown in Table 1.

Table-1. Volatile compounds composition of fresh squid sample

Groups		Retention Time (minutes)	Compounds	Area	Proportion (%)
Hydrocarbons	1	18,3583	1H-Indene, 1-methylene-	83442	6,49
	2	24,5201	Undecane	40608	3,16
	3	24,5118	Hexadecane	38225	2,97
	4	28,2876	Hexane, 2,3,5-trimethyl-	18208	1,42
	5	14,4786	1,3,6-Heptatriene, 5-methyl-	836	0,06
	6	37,4661	Toluene	285	0,02
Aldehydes	7	4,639	Acetaldehyde	2875	0,22
	8	39,3604	Pentanal	1248	0,10
Alcohols	9	7,6319	Silanediol, dimethyl-	1055682	82,07
	10	24,5018	1-Pentanol	1883	0,15
	11	15,8588	2-Hexen-1-ol, (E)-	662	0,05
Ketones	12	5,1732	2,3-Pentanedione	3634	0,28
Others	13	5,6555	Methylamine, N,N-dimethyl-	21889	1,70
	14	16,1327	3,6-Bis(N,N-dimethylamino)-9-methylcarbazole	13355	1,04
	15	16,0028	2,3-Dimethyl-aziridine	3450	0,27

The group of alcohol compounds (3 compounds) was the group with the highest proportion value identified in fresh squid samples. Dimethyl silanediol compound, has a proportion value of 82.07%. This compound was previously known to be found in fresh tiger shrimp samples as well as the compound with the highest proportion [14]. Dimethyl silanediol is a compound found in the environment and can contaminate through wastewater [15]. According to [16] this compound is referred to as the main contaminant in water. The group of alcohol compounds identified in fish according to [17] is formed from fat oxidation, fatty acids and amino acid degradation. [18] also stated that alcohol compounds are generally formed from the decomposition of secondary hydroperoxides of fatty acids. The results of this research also identified alcohol compounds which are generally not derived from

contaminants, namely 1-pentanol and 2-hexen-1-ol. Alcoholic compounds generally give fruity, oral and grassy effects to fish [7]. Volatile compounds from the alcohol group generally play a minimal role in the flavor of foodstuffs because of their high threshold unless these alcohol compounds are contained in high concentrations [19].

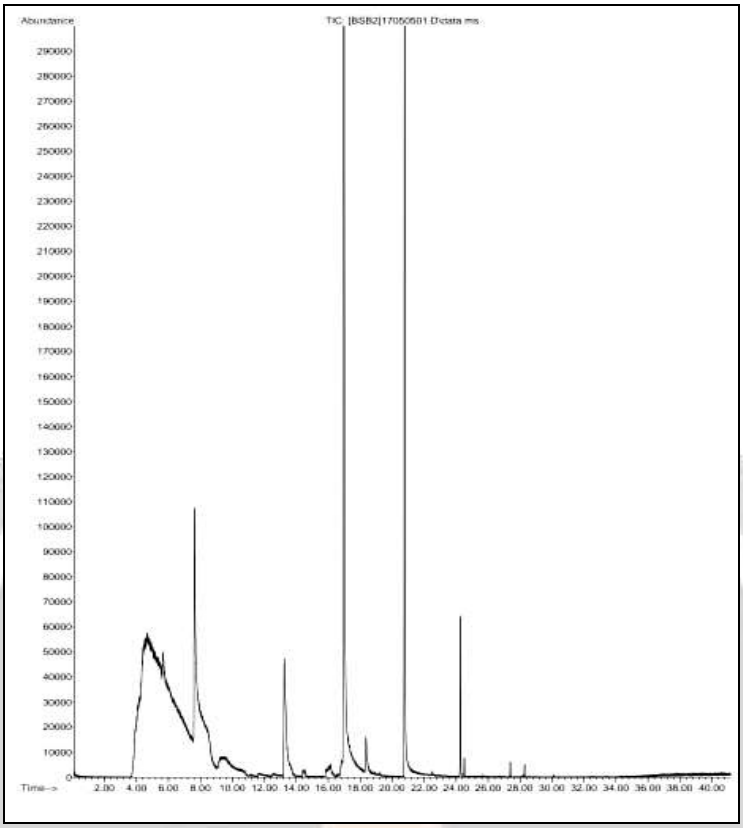

Fig-1. Volatile compounds chromatogram of fresh squid (*Loligo* sp.)

The hydrocarbon compounds detected in the fresh squid samples were 6 compounds with 1-methylene-1H-Indene as the compound having the highest proportion in this group, which was 6.49%. According to [20], 1-methylene-1H-Indene has a characteristic sour aroma. 1-methylene-1H-Indene is a cyclic chain hydrocarbon compound with the chemical formula $C_{10}H_8$. According to [7] some cyclic hydrocarbons identified in fish are the result of secondary reactions from thermal oxidation (heating) of carotenoids and other unsaturated fats.

The group of compounds identified in addition to the two groups above is the group of aldehyde compounds that were detected as many as 2 compounds with acetaldehyde as the compound having the highest proportion value of 0.22%. Acetaldehyde compounds are very volatile and if contained in high amounts it will give a green, grassy, apple-like aroma [21]. The detected aldehyde group compounds can come from the oxidation of the double carbon bonds of unsaturated fatty acids or saturated fatty acids [7]. The aroma threshold of carbonyl compounds is generally lower than that of alcohols [22], [23]. Therefore, the aldehyde group has a large influence and may interfere with other flavor compounds even in small amounts [24]. Most of the aldehyde group compounds that are known to have aromas such as green plants, grassy, dark chocolate, malty, fatty, sweet floral, such as apples, such as melon, nutty, and fruity and are found in various fresh fish and shellfish at various concentrations [24], [25], [26], [27].

Compounds from the ketone group only identified 1 compound, namely 2,3-Pentanedione. In the food flavor industry, 2,3-Pentanedione compounds can provide a butter-like aroma quality [28]. Ketone group compounds can be formed as a result of the oxidation of fats and fatty acids as well as the degradation of amino acids during the processing [29]. According to [7] compounds of the ketone group can be produced from thermal oxidation and degradation of unsaturated fatty acids, amino acid degradation or oxidation by microorganisms.

In addition to the groups of compounds that have been mentioned, there are groups of compounds that are not commonly identified in samples of fresh and steamed fishery products, these compounds are classified as other

groups of compounds. The compound that has the highest proportion in this group of compounds is N,N-dimethyl-methylamine. N,N-dimethyl-methylamine has another name trimethylamine [30]. According to [31] this compound is one of the compounds that has the potential to be used as an indicator of meat quality.

3.2 Proximate Analysis

The results of the proximate test of fresh squid can be seen in Table 2. The difference in the results of this measurement can be influenced by the initial chemicals composition content of the raw materials, the type of each commodity that is being analyzed and the processing process that has been given [8].

Table-2. Proximate content of fresh squid samples

Parameters	Value (%)
Moisture	84.01 ± 0,007
Ash	1.01 ± 0,00
Fat	0.65 ± 0,06
Protein	14.13 ± 0,007

Fresh squid samples contain 84% moisture content. Lower value of moisture content value was produced by the study of [32]. According to [32] the moisture content of raw squid (*Loligo* sp.) is 76.7%. Differences in measurement results can be caused by different methods of sample preparation, species, habitat of the samples used. According to [33], differences in water content can be caused by species, age of biota, differences in environmental conditions, and the level of freshness of organisms. Fresh squid samples contain 1.01% ash content. This result is not much different from the ash content value in [32] research where according to their research the ash content of raw squid (*Loligo* sp.) was 1.6%. The ash content in this product is more influenced by the mineral content compared to the processing stage of the raw material. The ash content in the sample originated from the mineral content present in the squid's meat and not from the addition of other ingredients. Squid has a very high and varied content of micro and macro minerals. This variation depends on the environmental conditions in which they live, size and age [34].

Fresh squid samples contain 0.65% fat content. This result is not much different from the research of [32] where raw squid (*Loligo* sp.) has a fat content of 0.8%. According to [12], the water content will affect the value of the fat content (and other nutrient levels) measured in the proximate test. The fat content in fishery commodities found in meat directly affects the intensity of aroma and taste [6]. Fresh squid samples contain 14.1% protein content. This measured value is lower than the research conducted by [32] where the protein content of raw squid (*Loligo* sp.) was 19.5%. [35] states that the measured protein content depends on the amount of ingredients added and is largely influenced by the water content of the ingredients.

4. CONCLUSIONS

Based on the results and discussion above, it can be concluded that the total compounds detected in fresh squid samples were 15 compounds. The volatile flavor compounds identified were from the hydrocarbon group as many as 6 compounds, aldehydes as many as 2 compounds, alcohol as many as 3 compounds, ketones as many as 1 compound, and 3 compounds categorized as others group. The dominant volatile flavor compound in fresh squid is dimethyl silanediol. The proximate content of fresh squid consist of 84.01% moisture content, 1.01% ash content, 0.65% fat content and 14.13% protein content.

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