Volatile components of raw Patin Catfish (Pangasius hypophthalmus) and Nile Tilapia (Oreochromis niloticus)

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Abstract. Volatile components are groups of compounds which could affect the aroma and acceptance of commodities. The objective of this research was to identify volatile components of two most common freshwater commodities in Indonesia which are patin catfish (Pangasius hypophthalmus) and nile tilapia (Oreochromis niloticus). The method used in this study were to detect volatile compounds using Gas Chromatography/Mass Spectrometry (GC/MS) on both fresh fish samples with Solid Phase Micro Extraction (SPME) as an initial extraction method. The volatile components analysis successfully detects and identify as many as 46 volatiles compounds in raw nile tilapia and 59 compounds were detected in raw patin catfish. Most of the detected components came from hydrocarbons, aldehydes, alcohols and ketone groups. Most of those volatile compounds detected were derived from the results of enzymatic reactions, lipid auto oxidation and various environmental influences.

1. Introduction
West Java Province, Indonesia has great potential in freshwater fisheries resources and its production has steadily grown over the year. Aquaculture plays an important role in providing fish supply nationally. Among West Java main production in aquaculture freshwater fish commodities are patin catfish (Pangasius hypophthalmus) and nile tilapia (Oreochromis niloticus). Most of nile tilapia production in West Java came from Purwakarta, Cianjur and West Bandung Districts and mainly cultured in ponds and floating net cage. In 2010, national nile tilapia production had reached 912,613 tons with 29.97% average increase in 5 years. Patin catfish is considered as one of the important freshwater fish export commodity in Indonesia among others. Patin catfish production has also shown a significant growth in number over the year. Its national production was 403,133 tons in 2014 with average increase as much as 39.90% [1] and in West Java, patin catfish are mainly coming from the floating net cage in Cirata and Jatiluhur Reservoir.

Fresh seafood is more susceptible to deterioration than other foods. Fresh fisheries commodities are a valuable nutritional source for protein and lipids, especially essential amino acids and polyunsaturated fatty acids. Each fishery commodity would have differences in their chemical composition and flavour compounds and that included their volatile components. Flavor characteristics and thus its components are an important determinants of food quality. Identifying flavor components in various foodstuff including fish and seafood that characterize them is important to carry out because flavor in general could influence sensory characteristics, consumption level, overall acceptance and choices of the end product by its consumers. The flavor is formed as a result from a combination of experiences and sensation which we perceived on product characteristics [2]. The flavour is generally divided into two categories, one is volatile flavour which contributes to aromas and other is non-volatiles flavour which contributes to taste characteristics. Volatile components of the flavor normally originate from within the raw material composition and its compounds are significantly affecting
product aroma. Volatile compounds in fishery commodities generally derive from various chemical compound groups such as hydrocarbon, aldehydes, alcohol, ketones, sulfurous and nitrogenous compounds, heterocyclic and esters [3,4].

Volatile compounds can be absorbed from the water, during fish respiration and then stored under the fish skin in the lipid layer. Lipid derived volatile compounds such as aldehydes and ketones are frequently generated by oxidative enzymatic reactions and autooxidation of lipids. Formation of volatile compounds derives from fatty acid oxidation leading to specific aromas could also be seen as an opportunity to develop and enhance the sensory quality of food product containing fish or seafood [5]. Volatiles flavors are easily evaporating therefore the precaution of handling and transporting the raw material or product are becoming utmost important. The production of volatiles in seafood is influenced by several factors such as species, food parts, environment, ripeness, biological factors, processing and other conditions. Some distinctive volatiles have been used to identify the freshness, deterioration and transformed seafood, and to evaluate the rearing water quality and the geographical origin [6].

In several countries such as China, Japan and Scandinavian countries, the study of fishery commodities volatile components were already carried out for more than a decade ago with various fish species such as carp, pond smelt, loach and silver carp [4,7], sea bream, black bream [8,9], rainbow trout [9], scallop [10], and salmon [11]. To our best knowledge, there was still no comprehensive study found in Indonesia that has been conducted in investigating the volatile components of raw patin catfish and nile tilapia.

The results of this research will be useful in the fisheries flavor research field for example, in conducting volatile components mapping to identify the key component which could influence aroma characteristic of certain fish species and also beneficial for various flavor studies application such as storage quality parameters and in producing flavor extract derived from fishery commodity. Therefore an attempt on volatile flavor analysis of two common fishery commodities is important to carry out. The objective of this research is to identify the volatile compounds of two main aquaculture in Indonesia which are raw patin catfish and nile tilapia.

2. Research method

Patin catfish and nile tilapia samples were taken from floating net cage complex in Cirata Reservoir, Purwakarta District, West Java. Sample preparation was carried out in the Fisheries Product Processing Laboratory, Fisheries and Marine Sciences Faculty, Padjadjaran University and volatile compounds analysis were carried out in Flavor Laboratory, Indonesian Centre for Rice Research, Sukamandi, Subang.

2.1. Samples preparation

After sampling, all samples were then promptly transported in a cool box which contained layers of bulk ice and transported to the laboratory. Samples were then washed, descaled, beheaded and weighed. Each fish samples were then filleted. A portion of fish meat (white meat) samples from the fillet were selected and weighed sufficiently for volatile analysis. All fish samples were then labelled and packaged in three different packaging layers. The first primary packaging was aluminium foil, the secondary packaging was cling wrap plastics and the tertiary packaging is a zip-lock plastic bags. The purpose of this layered packaging is to minimize the changes and degradation that could happen to the samples during transportation to the analysis laboratory which could be caused by various environmental factors such as air, light and temperatures [12]. Samples transported were placed inside a cool box which contain sufficient amount of packed ice to keep the samples dry and maintaining cool temperatures.

2.2. Volatiles compound analysis

Volatile component from both fish species were analyzed according modification of [9] procedure. The analysis was carried out using waterbath for samples extraction and Gas Chromatography (GC) (Agilent Technologies 7890A GC System) and Mass Spectrometry (MS) apparatus (Agilent Technologies 5975C Inert XL EI CI/MSD) for detecting and identifying the volatile components.
Samples extraction method was done by Headspace Solid Phase Micro Extraction (HS/SPME) using DVB/Carboxen/Poly Dimethyl Siloxane fiber. Sample extraction time used on waterbath was 40°C for 45 minutes. GC column used was HP-5MS (30 m x 250 μm x 0.25 μm), helium carrier gas, initial temperature was 45°C (hold 2 minutes), temperature escalation as much as 6°C/minutes, final device temperatures 250°C (hold 5 minutes) with an overall running time 41.17 minutes.

2.3. Data analysis

Samples volatile components mass spectrums detected from GC/MS were then compared with the mass spectrum pattern which were available in computer database or NIST (National Institute of Standard and Technology) library 0.5a version. The data then were further analyzed with Automatic Mass Spectral Deconvolution and Identification System (AMDIS) software [13]. The resulting data from volatile compound analysis were discussed descriptively based on identification and the semi quantification intensity of the compounds detected from the analyzed samples.

3. Results and discussion

Volatile compounds analysis results showed that raw patin catfish has higher quantities of volatile compounds compared to the compounds that were identified from raw nile tilapia samples. Wide variation of compounds was also observed from both species. Volatile compounds analysis results from raw patin catfish samples successfully detected and identified as much as 59 volatile compounds and from raw nile tilapia as many as 46 compounds. The compounds detected were then categorized into several major groups such as hydrocarbon, aldehydes, alcohols, ketones and others which categorized as nitrogenous and furan compound groups.

Among 59 identified volatile components in raw patin catfish, the dominant five volatiles were hexadecane, hexanal, 1-octen-3-ol, 2,3-octadione and nonanal, whereas in nile tilapia were hexanal, 6-methyl-3-heptanone, nonanal, heptanal and 2-decanone. The compound with the highest proportion (%) in raw patin catfish originated from hydrocarbon groups (aliphatic, cyclic and aromatic) which is hexadecane (23.904%), however aldehyde group has the highest compounds variety (20 compounds) with hexanal as the most abundant compounds in this group (16.978%) followed by hydrocarbons (17 compounds). In addition to hydrocarbons and aldehydes, GC/MS have also detected alcohol group (14 compounds) with 1-octen-3-ol (14.317%) that has the highest proportions and ketones (6 compounds) with 2,3-octanedione (10.333%) that has the highest proportions. Volatile component analysis had also detected two furan compounds, 2-pentylfuran previously detected in steamed silver carp and smoked black bream [4,9] and 2-ethylfurran. Furan compounds are heterocyclic and usually derive from glucose dehydration (cellulose thermal degradation), but several of them could also derive from Maillard reaction [14,15].

Volatile compounds analysis result from raw nile tilapia samples successfully detected as many as 46 compounds which then were categorized into several major groups such as hydrocarbon, aldehydes, alcohols, ketones and others. Aldehydes in raw nile tilapia samples was found to be the highest in quantity (16 compounds) with hexanal as the most abundant compounds (43.066%). Similar results were found in [16] regarding study on raw tench fish which showed two major categories of volatile compounds identified and they were aldehydes (21 compounds) and alcohols (6 compounds). In addition to aldehydes, GC/MS have also detected hydrocarbon group (10 compounds) with pentadecane (2.395%) has the highest proportions, alcohols (10 compounds) with 1-octen-3-ol (2.684%) has the highest proportion, ketones (8 compounds) with 6-methyl-3-heptanone (23.527%) has the highest proportion. The volatile component analysis was also detected one nitrogenous compound (N,N-dimethyl-methylamine) and one furan group compound. Volatile compounds analysis results for raw patin catfish and for raw nile tilapia are shown in table 1 with their proportions (based on area percentage).
Table 1. Volatile compounds in raw patin catfish and nile tilapia samples.

| Groups                              | Raw Patin catfish | Raw Nile tilapia |
|-------------------------------------|-------------------|------------------|
|                                     | Area  | Proportion (%)  | Area  | Proportion (%)  |
| **Hydrocarbons (aliphatic, cyclic, aromatic)** |       |                 |       |                 |
| Toluene                             | 39992 | 0.016           | -     | -               |
| 1-Hexene, 3,5,5-trimethyl-           | 6765244 | 2.631          | -     | -               |
| Benzene, 2-ethyl-1,4-dimethyl-       | 797887 | 0.310           | -     | -               |
| Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)- | 348133 | 0.135           | 59013 | 0.070           |
| 1,3,6-Heptatriene, 5-methyl-         | 303507 | 0.118           | 33927 | 0.040           |
| 1,4-Cyclooctadiene, (Z,Z)-          | 97076  | 0.038           | -     | -               |
| Undecane                            | 812821 | 0.316           | 531167| 0.632           |
| Naphthalene                         | 1076584 | 0.419          | -     | -               |
| 2-Decanone                          | 1263020 | 0.491          | -     | -               |
| Pentadecane                         | 8274270 | 3.218           | 2013723 | 2.395 |
| Hexadecane                          | 61467667 | 23.904         | -     | -               |
| Heptadecane                         | -     | -               | 1209960 | 1.439 |
| Nonadecane                          | -     | -               | 106768 | 0.127           |
| Tridecane                           | 1999636 | 0.778           | 313326| 0.373           |
| Tetradecane                         | 2060130 | 0.801           | 554156| 0.659           |
| 8-Heptadecene                       | 1339404 | 0.521           | -     | -               |
| 1-Nonadecene                        | 70830  | 0.028           | -     | -               |
| Hexadecane                          | 1806020 | 0.702           | 290366| 0.345           |
| Dodecane                            | 835765  | 0.325           | 340471| 0.405           |
| **Aldehydes**                       |       |                 |       |                 |
| Butanal                             | 1539386 | 0.599           | 1681918 | 2.000 |
| Pentanal                            | 43657045 | 16.978          | 36214661 | 43.066 |
| Hexanal                             | 71176  | 0.028           | 43815  | 0.052           |
| 2-Hexenal, (E)-                     | 3172954 | 1.234           | 5728   | 0.007           |
| Heptanal                            | 9021083 | 3.508           | 4505298 | 5.358 |
| 2-Heptenal, (E)-                    | 151174  | 0.059           | 150939 | 0.178           |
| Tridecanal                          | 3283716 | 1.277           | -     | -               |
| Octanal                             | 45860  | 0.181           | -     | -               |
| 2-Octenal, (E)-                     | 519663  | 0.202           | 278148 | 0.331           |
| 2,4-Heptadienal, (E,E)-             | 168136  | 0.065           | 12256  | 0.015           |
| Nonanal                             | 13993674 | 5.442           | 4746845 | 5.645 |
| 2,6-Nonadienal, (E,Z)-              | 409543  | 0.159           | 177678 | 0.211           |
| Benzaldehyde, 4-ethyl-              | 282252  | 0.110           | -     | -               |
| Decanal                             | 722177  | 0.281           | 571619 | 0.680           |
| 2-Undecenal                         | 412303  | 0.160           | 31930  | 0.038           |
| 2-Nonenal, (E)-                     | 183830  | 0.071           | 300092 | 0.357           |
| 2,4-Hexadienal, (E,E)-              | 219346  | 0.085           | -     | -               |
| Tetradecanal                        | 630001  | 0.245           | -     | -               |
| Hexadecanal                         | 1319190 | 0.513           | 106734 | 0.127           |
| Dodecane                            | 255578  | 0.099           | 60201  | 0.072           |
| **Alcohols**                        |       |                 |       |                 |
| 2-Penten-1-ol, (E)-                 | 950089  | 0.369           | -     | -               |
| 2-Penten-1-ol, (Z)-                 | 351592  | 0.137           | -     | -               |
| 1-Hexanol                           | 2193194 | 0.853           | 826277 | 0.983           |
| 1-Octanol                           | 82325  | 0.032           | 1078548 | 1.283 |
| 1-Octen-3-ol                        | 36814527 | 14.317          | 2257040 | 2.684 |
| 2-Octen-1-ol                        | 3895611  | 1.515           | 140399 | 0.167           |
In general, fresh fish are characterized by sweet, mild, green, plant-like, metallic and fishy aromas and volatile compounds contributing to these aromas are generated mainly by oxidative enzymatic reactions and auto oxidation of lipids [7]. According to [16], the flavor associated with freshwater fish is usually mild, delicate and pleasant and most fish have a common sweet and plant-like aroma. This fresh fish flavor is due to volatile aldehydes and alcohols which are mainly derived from the oxidative deterioration of polyunsaturated fatty acids. Based on panelists sensory evaluation, aroma description range of fresh patin catfish are fresh, neutral and has species specific aroma [17] while there are still no information found for raw nile tilapia sensory evaluation.

Wide variation of volatile compounds detected on raw patin catfish and nile tilapia in general were derived mainly from proteins and lipid content, thus the quantities and type of volatile compounds are related to chemical composition contained in both samples. Most of the volatile compounds were derived from the results of enzymatic reactions, microorganism activities, lipid auto oxidation, resulting substance from various thermal involved reactions and environmental impacts [8]. Several studies have been published regarding volatile components in fishery commodities, among them were [4,7,8,9,16,18,19,20,21]. The differences observed on volatile compounds might reflect the different volatile extraction methodologies used, the different species, age, part of the sample, biological factors and/or geographical origin of the samples [6].

In general, it can be assumed that raw patin catfish possessed a slightly higher number of volatile components compared to nile tilapia. One of the reasons is that the further increasing of such components was a result of biochemical pathways of protein and lipid of fish. Other factors that could affect the numbers and types of compounds detected in this volatile compounds analysis were extraction method (including time and temperature of extraction), type of samples and GC/MS column and its running parameters [20].

According to [4,10,14], hydrocarbon compounds groups detected in both samples could derive from decarboxylation reaction and the splitting process of fatty acids carbon chains, a secondary reaction from carotenoid (if present) and unsaturated fatty acids thermal oxidations. Aldehydes group compounds detected could derived from fatty acids carbon double bonds oxidation whether they were
saturated or unsaturated [4,9,10,22,23,24]. The odor thresholds of aldehydes are generally lower than those of other volatile compounds, thus they have a great potential effect on total flavor [16].

Almost all reaction that could produce volatile compounds would involve saturated and unsaturated fatty acids, which present in most of fishery commodities. Patin catfish has lipid content range from 0.89-1.23%. These results were not including the patin catfish belly parts. The belly parts of patin are known to contain high lipid content [17]. Lipid content in fish flesh directly affect odor and flavor intensity [25,26]. According to [27], nile tilapia has a broad fatty acid composition, including saturated, monounsaturated and polyunsaturated fatty acids. Its myristic acid and oleic acid content are relatively high with fatty acid profile consists of 45% saturated fatty acids, 13.9% monounsaturated fatty acids and 16.2% are polyunsaturated fatty acids.

Some of the hydrocarbons, aldehydes, alcohols and ketone groups which detected in samples such as hexadecane, nonadecane, tridecane, dodecane, 1-nonadecene, naphthenalene, toluene, nonanal, hexadecanal, pentanal, heptanal, 1-octen-3-ol, 1-nonanol, 1-hexanol, 2-ethyl-1-hexanol, 2-heptanone, 2-decanone were also known being detected in raw, cooked and recooked silver carp [4], wild and cultured sea bream [8] and raw black bream [9]. Heptane, tridecane, pentadecane were previously detected in squid volatile components. Hydrocarbons are generally considered to have less impact on the overall flavor of foods because their high aroma thresholds. The presence of the aromatic compounds such as naphthalene in fresh seafood could be associated with fuel pollution in the ocean [6].

Aldehydes such as hexanal was detected in both fish species and are known to have green-like flavour characteristic, pentanal was detected in both fish and has green flavour, (E,E)-2,4-heptadienal has cinnamon-like/oxidized oil-like and detected in both fish, (E)-2-octenal has cinnamon like flavour and detected in both fish, octanal were detected in raw catfish and has citrus like flavour characteristics, (E,Z)-2,6-nonadienal has cucumber like/insect like flavour characteristics and detected in both fish. Short chain volatile compounds such as E-2-nonenal that have been found in both fish were previously detected in grey mullet fish and have been related to rancid and bitter taste in fish. Aldehydes, specifically Strecker degradation derives, might impart nutty/malty nuances to the product while heptanal, octanal or nonanal might impart a more characteristic fishy flavor [5], [28]. Linoleic acid is a fish oil component and a precursor of hexanal. Octanal and nonanal might possibly derive from the high amount of oleic acid. EPA and DHA are the leading precursor for the development of oxidation products such as 4-heptenal and 2,4-heptadienal, that derive mainly from the oxidation of linoleic and linolenic acid, both of the compounds were detected in raw patin catfish and nile tilapia. Aliphatic aldehydes are prone to produce fishy or rancid type odors, such as (Z)-4-heptenal, hexanal and (E,E)-2,4-heptadienal [5].

The odor description of volatile aldehydes formed by autooxidation of n-3 polyunsaturated fatty acid (PUFA), such as 2-pentenal, 2-hexenal and 2,6-nonadienal, are generally pleasant and associated with green, cucumber, apple, mushroom and grass and 2,4-heptadienal is associated with green, cucumber but also oily and fatty notes. The odors of volatile aldehydes derived from n-6 PUFA, such hexanal, 2-octenal, 2-decenal and 2,4-decadienal are described as tallowy, fatty, herbaceous, nutty, oily and associated with fat-fried, cod oil and oxidized oil. The differences in the fatty acid composition were responsible of different volatile compound profile and different sensory characteristics of the fish fillet [16]. Benzaldehyde which detected in raw patin catfish is known for its almond/fruity/creamy/nutty aroma [7]. Aldehydes could provide significant aromas, either pleasant or rancid, to foodstuffs, and their odor threshold values are usually lower than those of alcohols. Thus, even trace amounts of aldehydes might override the flavor effect of some other substances [6].

Alcohol compound 1-penten-3-ol that was detected in all samples is responsible for the flavor of fresh marine products and generated from polyunsaturated fatty acid [28]. Volatile alcohols that were detected in both fish samples such as 1-nonanol has little impact on food flavors because of their high odor threshold, unless they are unsaturated or present at high concentrations [6]. Branched chain alcohols are formed by the secondary decomposition of hydroperoxides of the n-3 and n-6 PUFA. Although alcohols have relatively high odor threshold values, unsaturated alcohols such as 1-octen-3-ol, with usually lower threshold values, are expected to have higher impact on the overall aroma [5], [29] and individually 1-octen-3-ol has mushroom odor description [30].
Ketone compound 2,3-pentanedione that was detected in raw patin catfish were previously detected in fresh sardine \[18\], turbot, mussel, clam are produced by oxidation of \(n\)-3 PUFA \[30\] and has a caramel like flavour characteristic \[18\]. Generally, ketone group that presents in samples are known to contribute to the sweet aroma of many crustaceans \[7\]. Ketones together with aldehydes are the main products of lipid autooxidation of fatty acids or the auto-oxidation of unsaturated fatty acids via hydroperoxides. In addition, they can also produce as a secondary product from the Strecker reaction in Maillard reaction systems. Similar to aldehydes and ketones, alcohols may be formed by secondary decomposition of hydroperoxides of fatty acids \[5,29\]. However, they have also been attributed to enzymic peroxidation of \(n\)-3 and \(n\)-6 polyunsaturated fatty acids \[5\]. Ketones are derived from enzymatic degradation of poly-unsaturated fatty acids, amino acids or microbial oxidation. 2,3-pentanedione and 3,5-octadien-2-one (detected in both fish) also detected in squid, are the intense odors in squid and the latter has been reported as a lipid oxidation product in seafood. Several ketones might contribute to the cheesy odor of fish sauce \[6\]. Furans compound that were detected in raw patin catfish and nile tilapia could contribute to the flavor and aroma of fish products, although they have been found in raw fish, they are mainly derived from Maillard reaction after drying or grilling \[5\]. Furans can also be produced by oxidation of fatty acids as 2-pentyl furan (detected in both fish samples) that impart reversion, beany, grassy, and licorice-like flavors in soybean oil \[29\].

4. Conclusion
The detected volatile compounds from samples could be categorized into several groups which are hydrocarbons, aldehydes, alcohols, ketones and others (nitrogenous compounds and furans). As many as 59 volatile compounds were detected and identified from raw patin catfish samples and 46 volatile compounds were from raw nile tilapia. It is shown from these results that raw patin catfish has a slightly higher quantity of volatile compounds compared to the compounds that were identified from raw nile tilapia samples. Hexadecane is being the most abundant proportion compound detected in raw patin catfish while in raw nile tilapia, hexanal has the highest proportion. The volatile component information reported in this research could provide important basic information and be further exploited in more advance flavor research and applications.

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