Amino Acid Profile and Volatile Flavour Compounds of Raw and Steamed Patin Catfish (Pangasius hypophthalmus) and Narrow-barred Spanish Mackerel (Scomberomorus commerson)

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Abstract. Fish species and processing methods could affect the volatile flavour composition and amino acid profile of fishery commodity. The objectives of this study were to identify volatile components and amino acid profile of two considered predominant fish species in Indonesia which are freshwater Patin catfish (Pangasius hypophthalmus) and marine water fish, Spanish mackerel (Scomberomorus commerson). The methods used in this study were to detect volatile compounds using Gas Chromatography/Mass Spectrometry (GC/MS) on fresh and steamed of both species samples (100°C for 30 minutes) and amino acid profile were also analyzed using High Performance Liquid Chromatography (HPLC). The volatile components analysis successfully detects as much as 29 and 59 volatiles compounds in fresh and steamed Patin catfish respectively, while 37 and 102 compounds were detected in fresh and steamed Spanish mackerel samples. Most of detected components derive from hydrocarbons, aldehydes, alcohols and ketone groups which could affected by their chemical composition and resulted from various thermal involved reaction. The amino acids profile identification results showed that glutamic acid was found higher compared to other amino acids standards in both samples. Glutamic acid is non-essential amino acid which is important in umami taste substances.

1. Introduction

With its continuously growing population, Indonesia in the near future will face an increasing foodstuff demand, especially in fishery commodities, considering Indonesia has a vast coastal and ocean area. West Java province has the densest population in Indonesia, thereby developing freshwater and marine water fishery sectors as a source of food will become more important for this province. From 2003 to 2010 fish aquaculture in West Java were increasing from production volume of 230,523 to 622,961 ton [1]. Patin catfish (Pangasius hypophthalmus) is one of the strategic targets for aquaculture freshwater fisheries development in Indonesia, aside from dumbo catfish (Clarias gariepinus), nile tilapia (Oreochromis niloticus), common carp (Cyprinus carpio) and gourami (Osphronemus goramy) due to its high value as an export commodities and it is a commonly raised fish species in freshwater aquaculture. Patin catfish production has shown a significant growth in number. Its national production was 403,133 tons in 2014 with average increase as much as 39.90% [2]. On the other side, sea catch production in West Java has reached 180,402.14 tons in 2010. Sea catch commodities that were landed in West Java, generally consists of several groups of fish such as
pelagic and demersal fish and non-fish (crustacean and molluscs). Economically important fish in pelagic group are dominated by several fish species such as mackerel tuna, chub mackerel, narrow-barred Spanish mackerel, anchovy and big tunas. Spanish mackerel specifically had increased production as much as 17% in 2010, with a production value in West Java alone was 5,071 tons [1].

Fisheries commodities have long been recognized as a valuable nutritional source such as protein and lipids. Each fishery commodity would have differences in their chemical composition and flavour compounds, specifically volatile components, whether if it is still in its fresh or in already processed condition before it is consumed. Information about the chemical composition of a commodity is important to identify the nutritive values, chemical changes and its relation to certain handling, processing or storage method. Such information can also beneficial in order to study and evaluate the potential of a commodity.

Volatile components are groups of compounds that contribute to product flavours specifically aroma. As we all know flavour is an important factor which could affect product acceptance and preference by consumers. Flavour is formed as a result from a combination of experiences and sensation which we perceived on product characteristics [3]. Flavour is generally divided into two categories, one is volatile flavour which contributes to aromas and non-volatile flavour which contributes to taste characteristics.

Many researches have confirmed about the volatile components differences among fisheries products, several of them were [4], [5], [6], [7], [8], [9], [10], [11]. In Indonesia, similar research data concerning flavour composition of fisheries commodities were not easily available and rarely found. Different matters occurred in other countries such as Japan, China, and Scandinavian countries where flavour research, including its identification has been done for more than a decade ago. Many researches object in identifying volatile compounds that were found in Indonesia came from agricultural commodities, but for fishery commodities it is still scarce.

In addition to identifying volatile components on fresh samples, this research also identified volatile components in samples that had already been steamed. Steaming is one of the thermal processing methods which used saturated steam as its heating medium. Steaming could induce physical changes and chemical reactions which in turn could affect product characteristics such as flavour and texture [12]. Steaming process was chosen as processing technique applied due to its minimum impact in sample’s nutritional value and it is one of the commonly used household cooking method and traditional processing method in West Java cuisines for making “pepes” or traditional steamed fish with spices. According to [13], steaming process has an advantage compared to other heating methods, because it has smaller risks of losing heat sensitive vitamins and other food compounds. The flavour of processed fish and fishery products differ with the processing technology employed. Such differences exist, although the products prepared from the same species of fish. Therefore the study of flavour components of processed fish and fresh fishery commodities is required as an attempt to specify and identify flavour components of such processed fish [14].

The amino acid profile analysis could provide general information regarding essential and non-essential amino acid composition and also beneficial to indicate which one of the overall amino acids that could affect samples taste characteristics. Heating could cause chemical changes in the amino acid residues, which then later could modify the structural, digestible and functional properties of proteins depending on the applied thermal treatment and processing conditions. According to [15] amino acids and peptides contribute directly to seafood flavour. Non-volatile flavour compounds are usually originated from free amino acids, various peptides, nucleotides such as IMP (disodium 5′-inosine monophosphate), GMP (disodium 5′-guanosine monophosphate), and AMP (disodium 5′-adenosine monophosphate) [10], [16].

The volatile components and amino acids composition results from this research could provide important basic information for more advance flavour research and applications. Thus, the results are also expected to contribute in filling the literature gaps of flavour profile originate from Indonesian fisheries commodities. Therefore an attempt on identification analysis of volatile flavour composition and amino acids of two popular fishery samples is important to carry out. The objective of this
research is to identify flavour composition, particularly volatile compounds and amino acid profile of fresh and steamed Patin catfish and Spanish mackerels samples. To our best knowledge, there was still no comprehensive study found in Indonesia that has been conducted in investigating the volatile components of fresh and steamed local Patin catfish and Spanish mackerel.

2. Research Method
Research locations for sampling and analyses were consisting of several areas in West Java. Patin catfish samples were taken from floating net cage complex in Cirata Reservoir, Purwakarta, West Java and Spanish mackerel samples were taken from the fish landing site, Karangsong, Indramayu West Java. Sample preparation was carried out in the Fisheries Product Processing Laboratory, Fisheries and Marine Sciences Faculty, Padjadjaran University. Proximate analyses were carried out in the Inter-University Centre Laboratory, Bogor Agriculture Institute. Amino acid profile analyses were carried out in the Integrated Laboratory, Bogor Agriculture Institute and volatile compound analysis were carried out in Flavour Laboratory, Indonesian Centre for Rice Research, Sukamandi, Subang.

2.1. Samples preparation
After sampling had finished on each location, all samples were then promptly transported in a cool box which contained layers of bulk ice and depart to preparation laboratory. On arrival, samples were then cleaned (washing, descaling, beheading), and weighed before divided into two groups (fresh and steamed). The fish that are intended for fresh groups were filleted before packaging, and the fish that are intended for steamed groups were eviscerated first before steaming and packaging. The steaming process was carried out at (minimum) 100°C for 30 minutes [10], [17]. After all preparation and treatment were completed, a portion of fish meat (white meat portion) samples were selected and weighed adequately for analyses purpose.

All weighed portions of the samples were then packaged into three different packaging layers. The first primary packaging was aluminium foil, the secondary packaging was cling wrap plastics and the last layer tertiary packaging is a zip-lock plastic bags with labels. The purpose of three layered packaging is to minimize the changes and degradation to the samples that could be caused by various environmental factors such as air, light and temperatures [18]. Samples that have been finished through packaging steps were then placed in cool box which contain sufficient amount of ice (packed inside plastics) to keep the samples in cool temperature and then promptly transported to each analysis laboratory.

2.2. Proximate analysis
Each sample was then analyzed for their moisture, ash, protein and lipid content. The proximate analysis for all fresh and steamed Patin catfish and Spanish mackerel samples were determined according to [19] standard. Moisture was calculated gravimetrically after complete drying of samples in an oven at 110°C, and total inorganic content (ash %) through combustion of organic matter in a muffle furnace for 24 hours at 450°C. Total protein content was determined by the Kjeldahl method and calculated as % nitrogen x 6.25. Total lipid content was determined by the Soxhlet system by drying samples in an oven (105°C) and refluxed for 8 hours using 150 ml chloroform inside Soxhlet tube and the results expressed in %.

2.3. Amino acids profile
Amino acids profiles of fresh and steamed Patin catfish and Spanish mackerel were carried out based on modification from [20], [21] using High Performance Liquid Chromatography (HPLC) (Shimadzu CBM-20A, Shimadzu Corporation, Japan). HPLC parameters setting used, i.e.: Ultra Techsphere column, 1mL/minute mobile phase flow rate with a fluorescence detector.

2.4. Volatiles compound analysis
Volatiles component from fresh and steamed of both fish were analyzed by procedures according to modification from [22] procedure. The analyses were 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’s extraction time used on waterbath was 35°C for fresh samples and 70°C for steamed samples for 45 minutes. Raising temperature increased sensitivity while allowing extraction of more compounds, notably the least volatile of which were not extracted at all at lower temperatures [23]. 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’s escalation as much as 6°C/minutes, the final device temperature was 250°C (hold 5 minutes) with an overall running time 41,17 minutes.

2.5. Data analysis

The obtained results from proximate analysis samples were calculated and showed as mean value and its deviation standards and then were discussed descriptively. The amino acid profile results were identified using 15 standards of amino acids and quantified in μmol concentration unit based on their peak areas and amino acids standards peak areas.

Samples volatile components mass spectrums that were 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 [24]. 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

3.1. Proximate analysis

Proximate analysis provides information regarding sample’s chemical, nutritional compositions and studies their changes. Moisture content, ash, protein and lipid content of fresh and steamed Patin catfish and Spanish mackerel samples were analyzed and the results are shown in Table 1. The proximate composition of both fish were relatively differ. The determination of moisture content is the most frequent general analysis performed on fishery commodities. The amount of water or moisture in food often determines its nutritive value and taste, and its shelf life stability throughout storage [25]. Moisture content analysis showed that fresh samples of both fish were found to contain relatively higher moisture content than the steamed one. Fresh and steamed Patin catfish have 79.65% and 72.26% moisture content, respectively, and fresh and steamed Spanish mackerel have 74.63% and 68.72% moisture content respectively. Moisture content of fish would give an effect on its textural characteristics and if it is too high, then the fish would have a soft and mushy texture [26]. These differences on moisture contents could be influenced by the type of commodities that were analyzed and processing method that the samples had been through [17], [18]. According to [13], processing technique such as steaming could lead to moisture loss from samples inter-cellular spaces and this could become the reason for the lower measurement of steamed samples moisture content.

The analysis result also showed slight differences in steamed samples ash content of both fish species if compared to fresh one. Fresh and steamed Patin catfish have 1.30% and 1.87% ash content, respectively, and fresh and steamed Spanish mackerel have 2.23% and 2.11% ash content, respectively. Ash content measurement was mostly dependent on the mineral contents of each sample, feed, growth phase, seasons and habitat environment. Ash content represents the amount of total mineral of samples measured and inorganic substances that present in samples which were the residue from high temperatures burning of organic samples [17], [18]. According to [27], minerals that had been added to snake skin gourami and short-bodied mackerel when it was processed by salting and
sun-drying could result in a much higher ash content ranged from 4.4 to 8.7 g/100 g. This could happen depending of the type of products, amount of minerals added and the salting method used.

### Table 1. Proximate analysis of Patin catfish and Spanish mackerel (%)

| Parameters | Patin catfish | Spanish Mackerel |
|------------|---------------|------------------|
|            | Fresh         | Steamed          | Fresh | Steamed |
| Moisture   | 79.65±0.26    | 72.26±0.08       | 74.63±0.15 | 68.72±0.14 |
| Ash        | 1.30±0.02     | 1.87±0.07        | 2.23±0.04 | 2.11±0.01 |
| Lipids     | 1.01±0.02     | 1.75±0.04        | 0.17±0.04 | 0.24±0.06 |
| Protein    | 14.47±0.16    | 20.22±0.05       | 20.79±0.19 | 27.98±0.15 |

*data expressed in mean and its standard deviation, n=3*

From analysis result, we could see that steamed samples of both fish species have a slightly higher amount of total lipids compared to the fresh one. Fresh and steamed Patin catfish had 1.01% and 1.75% total lipid content, respectively, and fresh and steamed Spanish mackerel had 0.17% and 0.24% lipid content respectively. Similar findings on Patin catfish lipid content from [26] research which showed the lipid content range of 0.89-1.23%. These results were categorized as quite low and could be caused by filleting, which did not include the Patin catfish belly parts. The belly parts of Patin are known to contain high lipid content. These results were also consistent with [27] studies on the chemical composition of several Thai freshwater and marine water fish which showed that most of the marine fish measured had a lower lipid content compared to freshwater fish. The lipid content is found to be influenced by season and geographic location, with lower lipid content in fish from tropical waters. The low lipid content in marine fish is also attributed to environmental factors such as food supply and dietary sources. Water and fat losses during heat treatment could also contribute to the differences of lipid content measurement between fresh and steamed samples. Lipid content in fish flesh directly affect odor and flavour intensity [27], [28], [29].

Protein content analysis showed that steamed samples of both fish species had a higher amount of total protein compared to the fresh samples. Both samples were found to be rich sources of protein. Fresh and steamed Patin catfish had 14.47% and 20.22% protein content, respectively, while fresh and steamed Spanish mackerel had 20.79% and 27.98% protein content, respectively. Protein content in each sample would be affected by fish habitat, seasons, storage time and condition and also processing methods. Moisture content in the samples would also have a major effect on protein content measured in samples. Lower moisture content in the samples will result in higher protein content measured compared to fresh samples [28], [30]. Similar results were experienced on [27], protein content of raw short-bodied mackerel contains 21.1 g/100 g protein, while the steamed and fried one contained higher protein levels of 27.5 g/100 g.

### 3.2. Amino acids profile

The importance of amino acids in fish has been well established from the perspective of nutrition and fish flavours [31]. Amino acid composition will determine the quality of a protein which is among the most important macronutrients in human diet. The amino acid profile analysis could provide us valuable information regarding essential and non-essential amino acid composition which contained in analyzed samples. In addition to that, we could also obtain information regarding amino acids contribution in general to sample’s taste attributes. This research used 15 amino acids standards to quantify individual amino acids present in samples. These standards were aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, leucine, lysine and isoleucine. The fresh and steamed Patin catfish and Spanish mackerel were found to be relatively rich in amino acids. Their amino acid compositions are shown in Table 2 and overall the amino acid composition between fresh and steamed samples display slight variation in their values.
Table 2. Amino acids profile of Patin catfish and Spanish mackerel (%)

| Amino acids | Patin catfish | Spanish Mackerel |
|-------------|--------------|------------------|
|              | Fresh | Steamed | Fresh | Steamed |
| Aspartic acid | 1.98  | 3.41   | 2.23  | 2.88   |
| Glutamic acid | 3.28  | 5.50   | 3.61  | 4.63   |
| Serine       | 0.79  | 1.31   | 0.88  | 1.15   |
| Histidine*   | 0.45  | 0.86   | 1.12  | 1.26   |
| Glycine      | 0.81  | 1.48   | 1.09  | 1.48   |
| Threonine*   | 0.82  | 1.42   | 1.05  | 1.35   |
| Arginine     | 1.32  | 2.27   | 1.46  | 1.95   |
| Alanine      | 1.15  | 1.85   | 1.37  | 1.81   |
| Tyrosine     | 0.69  | 1.14   | 0.87  | 1.04   |
| Methionine*  | 0.61  | 0.84   | 0.74  | 0.82   |
| Valine*      | 1.06  | 1.93   | 1.30  | 1.69   |
| Phenylalanine* | 0.86 | 1.46   | 1.02  | 1.33   |
| Isoleucine*  | 1.06  | 1.84   | 1.21  | 1.58   |
| Leucine*     | 1.66  | 2.77   | 1.86  | 2.42   |
| Lysine*      | 1.72  | 3.20   | 2.37  | 3.01   |

*) essential amino acids

Essential amino acids that Patin catfish had from highest to lowest value were lysine, leucine, isoleucine, valine, phenylalanine, threonine, methionine and histidine. There are other types of essentials amino acid, which did not include in the analysis due to its standard was not available such as tryptophan. As the highest amount of essential amino acids contained in the samples, lysine plays an important role in the human body because it is needed as basic composition of blood antibody, strengthen the circulation and maintaining normal cell growth. Together with proline and vitamin C, lysine will decrease excessive blood triglycerides. Methionine is important for lipid metabolism, maintaining liver health, prevent lipid accumulation in the liver and the main artery, preventing allergy and osteoporosis [26]. Leucine is an important molecule that could stimulate the synthesis of muscle proteins and also has a therapeutic role in stress like trauma, burns, etc. Histidine is capable to perform multiple roles in human beings such as protein-protein interaction, precursor of histamine, an important neurotransmitter and also needed for the growth and repair tissues [32]. Deficiency in certain amino acids may hinder healing recovery process [33].

It can be seen from Table 2 that the steamed samples of both species were observed to possess a relatively higher level of amino acids than the fresh samples of both fish. Similar results on amino acid content were reported by [15] with drying treatment of fresh and dried squid samples. The time length of heat treatment and heating method also contributed to the change in amino acid contents of fish samples [33]. Proteolytic reaction that occurred during the heating process could cause free amino acids forming to increase [10], [34].

The highest amount of amino acids detected in fresh and steamed Patin catfish was glutamic acid (3.28%; 5.50%, respectively), whereas for fresh and steamed Spanish mackerels was also glutamic acid (3.61%; 4.63% respectively). The less glutamic acid contained in fish meat it would result in less savory taste of the fish meat [26]. Others amino acids that had a considerable amount in fresh and steamed Patin catfish samples respectively were aspartic acids (1.98%; 3.41%), lysine (1.72%; 3.20%), leucine (1.66%; 2.77%), arginine (1.32%; 2.27%), valine (1.06%; 1.93%), alanine (1.15%; 1.85%) and isoleucine (1.06%; 1.84%), whereas for fresh and steamed Spanish mackerel samples respectively were lysine (2.37%; 3.01%), aspartic acid (2.23%; 2.88%), leucine (1.86%; 2.42%) and arginine (1.46%; 1.95%) Leucine, valine, isoleucine and lysine are categorized as
essential amino acids on the account of that human body could not produce essential amino acids by itself and have to be obtained from various external food sources.

Each of the amino acids is well known to contribute to the basic taste of a product. Proline mainly contributes to the bitter taste of peptides and the presence of glycine, alanine, valine, leucine, tyrosine and phenylalanine in peptides also impart bitterness. Glutamate imparts umami taste if the concentration in the food product is above the taste threshold and it is likely that umami taste is a signal for protein nutrition [35], [36], [37], [38]. Arginine at sub threshold concentrations significantly enhanced salty taste [37] and give an effect to umami taste in sea urchin [36] at a large amounts in crab and scallops it enrich the sweet taste with complexity and fullness and provide a seafood flavour [35]. Glycine and alanine are taste active components and well known to impart sweetness characteristic in various seafood. Acidic L-amino acids such as glutamate and aspartate impart to umami taste and most of the D-amino acids are dominantly sweet [35], [36]. Aromatic amino acids, basic amino acids and branched amino acids are bitter amino acids. Valine, leucine and histidine are known to impart bitter taste but are not as bitter as phenylalanine [38]. Histidine was known to impart sourness and umami in katsuobushi [40]. According to [36], the amino acids that are present in a product played an important role in the taste of most seafood.

3.3. Volatile flavour components

Volatile compound analysis showed that steamed Patin catfish and Spanish mackerel samples has higher quantities of volatile compounds compared to the compounds that were identified from fresh samples and wide variety of compounds were also observed from both fresh and steamed Patin catfish samples. Volatile compound analysis results from fresh and steamed Patin catfish samples successfully detected and identified as much as 29 and 59 volatile compounds, respectively, which then were categorized into several major groups such as hydrocarbon, aldehydes, alcohols, ketones and others.

Aliphatic, cyclic and aromatic hydrocarbons in fresh and steamed Patin catfish samples (10 and 24 compounds, respectively) were the highest in quantity with heptadecane as the most abundant compounds present in fresh and steamed samples (29.923%; 35.661% respectively). In addition to hydrocarbons, respectively in the fresh and steamed Patin catfish samples, GC/MS were also detecting aldehydes group (7 and 18 compounds) with hexanal (10.130%; 4.963%) which had the highest proportions on both samples, alcohols (7 and 12 compounds) with 1-octanol (11.848%) and 1-octen-3-ol (4.604%) had the highest proportions, ketones (3 and 2 compounds) with 6-methyl-3-heptanone (6.086%) and 2,3-octanedione (1.114%) had the highest proportions in fresh and steamed samples respectively.

In the steamed samples, one ester compound was also detected, Sulfurous acid, dodecyl pentyl ester. Presumably, ester group compound that was found in fish samples, is derived from acids and alcohols esterification which previously formed from lipid metabolism. Esters could derive from lipids thermal degradation products [4], [22]. The volatile component analysis was also detected several nitrogenous group compounds which usually not detected in fresh and steamed fishery samples and need more rigorous identification and 2-pentylfuran which is also previously detected in steamed silver carp and smoked black bream [10], [22]. Furan compounds are heterocyclic and usually derive from glucose dehydration (cellulose thermal degradation), but several of them could also derive from Maillard reaction [4], [41]. The volatile compounds analysis results are shown in Table 3 and Table 4 with their proportions (based on area percentage) sorted from highest to lowest abundance.

### Table 3. Volatile compounds in fresh Patin catfish samples.

| RT      | Groups                               | Area  | Proportion (%) |
|---------|--------------------------------------|-------|----------------|
| 21.871  | Heptadecane (aliphatic, cyclic, aromatics) | 5251755 | 29.923        |
| 18.0407 | Pentadecane                           | 2433484 | 13.865        |
| 5.07    | Nonane, 3-methyl-                     | 1618664 | 9.223         |
Table 4. Volatile compounds in steamed Patin catfish samples.

| RT    | Groups                                   | Area       | Proportion (%) |
|-------|------------------------------------------|------------|----------------|
| 11.056| Naphthalene                              | 371604     | 2.117          |
| 5.595 | 3-Octene, 2,6-dimethyl-                  | 355593     | 2.026          |
| 20.001| Hexadecane                               | 346667     | 1.975          |
| 6.930 | Cyclohexene, 1-methyl-4-(1-methyletheny)-, (S)- | 186190     | 1.061          |
| 15.974| Undecane                                 | 162764     | 0.927          |
| 5.283 | Naphthalene, decahydro-                  | 54789      | 0.312          |
| 7.815 | 1,3,6-Heptatriene, 5-methyl-             | 47749      | 0.272          |
|       | **Aldehydes**                            |            |                |
| 2.536 | Hexanal                                  | 1777914    | 10.130         |
| 6.314 | 2,6-Nonadienal, (E,Z)-                  | 360119     | 2.052          |
| 9.213 | Nonanal                                  | 331775     | 1.890          |
| 5.423 | 2-Nonenal, (E)-                          | 144560     | 0.824          |
| 5.346 | 2,4-Hexadienal, (E,E)-                  | 118308     | 0.674          |
| 6.368 | Dodecanal                                | 114574     | 0.653          |
| 4.378 | Heptanal                                 | 5951       | 0.034          |
|       | **Alcohols**                             |            |                |
| 5.866 | 1-Octanol                                | 2079349    | 11.848         |
| 4.751 | 1-Nonanol                                | 34623      | 0.197          |
| 7.506 | 1-Hexanol, 2-ethyl-                      | 16602      | 0.095          |
| 5.382 | Heptanol                                 | 14889      | 0.085          |
| 4.724 | Hexanol                                  | 9910       | 0.056          |
| 11.663| 2-Hexen-1-ol, (E)-                      | 5996       | 0.034          |
| 19.291| 1-Penten-3-ol                           | 1810       | 0.010          |
|       | **Ketones**                              |            |                |
| 6.336 | 3-Heptanone, 6-methyl-                   | 1068060    | 6.086          |
| 6.331 | 2,3-Octanedione                          | 588120     | 3.351          |
| 13.705| 2-Heptanone                              | 15129      | 0.086          |
|       | **Others**                               |            |                |
| 23.665| 3-Methyl-5-hydroxy-isoxazole             | 32310      | 0.184          |
| 13.688| Methylamine, N,N-dimethyl-               | 1399       | 0.008          |
| (S)-trans-Calamenene | 474284 | 0.197 |
| Mesitylene | 330758 | 0.137 |
| Cyclohexene, 1-methyl-4-(1-methylethenyl)- | 489752 | 0.203 |
| 5.89 Nonadecene | 23625 | 0.134 |
| 1-Nonadecene | 323625 | 0.102 |
| 3-Carene | 245853 | 0.072 |
| 12.177 Bicyclo[5.1.0]octane, 8-(1-methylethylidene)- | 172844 | 0.064 |
| 2.1304 Toluene | 154794 | 0.004 |
| Undecane | 10486 | 0.004 |

**Aldehydes**

| Hexanal | 11966421 | 4.963 |
| Nonanal | 9702159 | 4.024 |
| Heptanal | 2573292 | 1.067 |
| Undecenal | 1534136 | 0.636 |
| Octanal | 1491872 | 0.619 |
| Decanal | 383382 | 0.159 |
| Dodecanal | 340726 | 0.141 |
| Pentanal | 299246 | 0.124 |
| Dodecanal | 279610 | 0.116 |
| Heptanal | 235256 | 0.098 |
| Dodecanal | 178670 | 0.074 |
| Heptadienal, (E,E)- | 139235 | 0.058 |
| Heptanal | 82835 | 0.034 |
| Nonenal, (E)- | 68753 | 0.029 |
| Butanal | 63014 | 0.026 |
| Nonadien, (E,Z)- | 32278 | 0.013 |
| Pentenal, (E)- | 24368 | 0.010 |
| Heptenal, (E)- | 6441 | 0.003 |

**Alcohols**

| 1-Octen-3-ol | 11100825 | 4.604 |
| Heptanol | 809845 | 0.336 |
| Octen-1-ol | 649970 | 0.270 |
| Hexanol | 273597 | 0.113 |
| Pentanol | 130539 | 0.054 |
| Nonadien-1-ol | 128579 | 0.053 |
| Penten-1-ol, (E)- | 66845 | 0.028 |
| Penten-3-ol | 58411 | 0.024 |
| Penten-1-ol, (Z)- | 29513 | 0.012 |
| Hexen-1-ol, (E)- | 19178 | 0.008 |
| Nonanol | 12390 | 0.005 |
| Octanol | 10931 | 0.005 |

**Ketones**

| 2,3-Octanedione | 2686472 | 1.114 |
| Heptanone | 10661 | 0.004 |

**Esters**

| Sulfurous acid, dodecyl pentyl ester | 255235 | 0.106 |

**Others**

| Indole | 1505303 | 0.624 |
| Furan, 2-pentyl- | 6295 | 0.003 |
Volatile compound analysis results from fresh and steamed Spanish mackerel samples successfully detected as much as 37 and 102 volatile compounds, respectively, which then were categorized into several major groups such as hydrocarbon, aldehydes, alcohols, ketones and others. Aliphatic, cyclic and aromatic hydrocarbons in fresh and steamed Spanish mackerel samples (12 and 42 compounds) were the highest in quantity with pentadecane as the most abundant compounds present in fresh and steamed samples (38.388%; 18.035% respectively). In addition to hydrocarbons, respectively in the fresh and steamed Spanish mackerel samples, GC/MS were also detecting aldehydes group (10 and 28 compounds) with hexanal (13.215%) and octanal (8.851%) which had the highest proportions, alcohols (9 and 16 compounds) with (Z)-2-Penten-1-ol (5.134%) and 1-octen-3-ol (4.315%) had the highest proportions, ketones (5 and 8 compounds) with 2,3-octanedione (6.103%) and 2-decanone (3.684%) had the highest proportions in fresh and steamed samples respectively. In the steamed Spanish mackerel samples, two ester compounds was also detected with (Z)-3-Hexenoic acid, methyl ester (0.149%) had the highest proportion. The volatile component analysis was also detected 6 compounds from various nitrogenous and furan groups compounds. The volatile compounds of fresh and steamed samples analyses results are shown in Table 5 and Table 6 with their proportions sorted from highest to lowest abundance.

| RT     | Groups                        | Area   | Proportion (%) |
|--------|-------------------------------|--------|----------------|
| 18.0164 | Pentadecane                   | 12194171 | 38.388         |
| 21.936  | Hexadecane, 2,6,11,15-tetramethyl- | 2601379  | 8.189          |
| 21.823  | Heptadecane                   | 1337526  | 4.211          |
| 11.038  | Naphthalene                   | 1099043  | 3.460          |
| 15.9526 | Tetradecane                   | 430556  | 1.355          |
| 19.9739 | Hexadecane                    | 351285  | 1.106          |
| 7.2736  | Limonene                      | 299447  | 0.943          |
| 13.7684 | Tridecane                     | 257358  | 0.810          |
| 11.4749 | Undecane                      | 110789  | 0.349          |
| 17.2373 | Heptane, 2,6-dimethyl-        | 83989   | 0.264          |
| 2.1406  | Toluene                       | 24093   | 0.076          |
| 7.3125  | Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)- | 11770  | 0.037          |
| 2.5391  | Hexanal                       | 4197730 | 13.215         |
| 4.3573  | Heptanal                      | 654198  | 2.059          |
| 9.1975  | Nonanal                       | 344298  | 1.084          |
| 6.0988  | 2-Octenal, (E)-              | 317901  | 1.001          |
| 13.5813 | 2-Nonenal, (E)-              | 135385  | 0.426          |
| 13.5818 | Butanal                       | 41607   | 0.131          |
| 11.6385 | Decanal                       | 19337   | 0.061          |
| 13.9591 | Dodecanal                     | 10359   | 0.033          |
| 14.7554 | Octanal                       | 2963    | 0.009          |
| 7.1832  | Benzaldehyde, 4-ethyl-        | 1834    | 0.006          |
| 6.2511  | 2-Penten-1-ol, (Z)-           | 1630891 | 5.134          |
| 6.2848  | 2-Octen-1-ol                 | 1012469 | 3.187          |
| 6.2529  | 1-Octen-3-ol                 | 844424  | 2.658          |
| 6.0846  | 1-Heptanol                    | 672161  | 2.116          |
| 1.3862  | 1-Penten-3-ol                | 186560  | 0.587          |
| 2.1843  | 1-Pentanol                    | 96996   | 0.305          |
| 2.4966  | 2-Hexen-1-ol, (E)-           | 77693   | 0.245          |
| 3.8367  | 1-Hexanol                     | 49894   | 0.157          |
| RT       | Groups                                                                 | Area  | Proportion (%) |
|----------|------------------------------------------------------------------------|-------|----------------|
| 7.4772  | 1-Hexanol, 2-ethyl-                                                     | 22449 | 0.071          |
|          | **Ketones**                                                            |       |                |
| 6.3249  | 2,3-Octanedione                                                        | 193881| 6.103          |
| 13.6716 | 2-Decanone                                                             | 421001| 1.325          |
| 1.4393  | 2,3-Pentanedione                                                       | 212674| 0.670          |
| 9.0534  | 3-Heptanone, 6-methyl-                                                 | 30247 | 0.095          |
| 8.9235  | 2-Heptanone                                                            | 10263 | 0.032          |
|          | **Others**                                                             |       |                |
| 6.4394  | Furan, 2-pentyl-                                                       | 32385 | 0.102          |

**Table 6.** Volatile compounds in steamed Spanish mackerel samples.

| RT       | Groups                                                                 | Area  | Proportion (%) |
|----------|------------------------------------------------------------------------|-------|----------------|
| 18.082   | Pentadecane                                                            | 156052623| 18.035        |
| 21.971   | Pentadecane, 2,6,10,14-tetramethyl-                                    | 66552586 | 7.691          |
| 21.853   | Heptadecane                                                            | 34976984 | 4.042          |
| 12.006   | Cyclohexene, 3-ethenyl-                                               | 12372341 | 1.430          |
| 13.7761  | Tridecane                                                              | 10019968 | 1.158          |
| 11.056   | Azulene                                                                | 8313380  | 0.961          |
| 19.9758  | Hexadecane                                                             | 8129937  | 0.940          |
| 15.9515  | Tetradecane                                                            | 7443583  | 0.860          |
| 11.4768  | Dodecane                                                               | 7109119  | 0.822          |
| 8.5505   | 1,3,6-Heptatriene, 5-methyl-                                           | 6705974  | 0.775          |
| 13.163   | 1,4-Octadiene                                                          | 5434787  | 0.628          |
| 5.418    | Cyclobutane, 1,2-diethenyl-                                            | 4767538  | 0.551          |
| 13.393   | 1,3-Cyclooctadiene, (Z,Z)-                                            | 4205079  | 0.486          |
| 9.0718   | Undecane                                                               | 3731451  | 0.431          |
| 8.653    | 3-Octyne                                                               | 2869724  | 0.332          |
| 17.237   | Heptadecane, 2,6,10,14-tetramethyl-                                    | 2453175  | 0.284          |
| 17.609   | 2-Tridecene, (E)-                                                     | 2418128  | 0.279          |
| 12.791   | 1,2-Heptadiene                                                         | 2287224  | 0.264          |
| 9.615    | Bicyclo[4.1.1]oct-2-ene                                               | 2207940  | 0.255          |
| 12.662   | Oxiirane, 3-butenyln-                                                  | 2113021  | 0.244          |
| 9.863    | Cyclopentane, 1-ethyl-3-methyl-, cis-                                  | 1885844  | 0.218          |
| 17.868   | 1-Pentadecene                                                          | 1879303  | 0.217          |
| 15.755   | Z-1,9-Hexadecadiene                                                   | 1765776  | 0.204          |
| 7.231    | β-Cymene                                                               | 1708841  | 0.197          |
| 9.45     | 1,3-Cyclooctadiene                                                    | 1690275  | 0.195          |
| 12.154   | Cyclopentane, 2-ethyl-1,1-dimethyl-                                    | 1608641  | 0.186          |
| 7.555    | 2,6-Octadiene, (E,E)-                                                 | 1373570  | 0.159          |
| 10.837   | Dispiro[2.1.2.1]octane                                                | 1301766  | 0.150          |
| 7.312    | Limonene                                                               | 1188173  | 0.137          |
| 15.235   | Bicyclo[10.1.0]tridec-1-ene                                           | 958734   | 0.111          |
| 8.754    | Cyclooctene, 3-ethenyl-                                                | 890796   | 0.103          |
| 21.443   | 3-Heptadecene, (Z)-                                                   | 675850   | 0.078          |
| 25.2988  | Nonadecane                                                             | 487865  | 0.056          |
| 19.834   | Z-8-Hexadecene                                                         | 474294  | 0.055          |
| 7.372    | 4-Decyne                                                               | 348494  | 0.040          |
| 23.491   | Nonadecene                                                             | 282882  | 0.033          |
| 13.558   | Tridecane, (Z)-                                                       | 275222  | 0.032          |
| 14.551   | Benzene, 1-methoxy-4-pentyl-                                           | 152838  | 0.018          |
| 19.5383  | Naphthalene, decahydro-                                               | 99718   | 0.012          |
| 17.75    | Cyclopentadecane                                                       | 90797   | 0.010          |
| Compound                          | Retention Time | Relative Intensity |
|----------------------------------|----------------|--------------------|
| Toluene                          | 2.1726         | 0.003              |
| Octahydro-1-oxo-cyclopropa[c]indene | 17.461         | 0.001              |

**Aldehydes**

| Compound                          | Retention Time | Relative Intensity |
|----------------------------------|----------------|--------------------|
| Hexanal                          | 6.7872         | 8.851              |
| Nonanal                          | 9.2394         | 8.670              |
| Hexanal                          | 2.6301         | 5.648              |
| Heptanal                         | 4.433          | 3.728              |
| Decanal                          | 11.6468        | 1.309              |
| Undecanal                        | 13.966         | 0.958              |
| Benaldehyde, 4-ethyl-             | 10.6137        | 0.788              |
| Tetradecanal                     | 20.245         | 0.780              |
| Dodecanal                        | 16.1723        | 0.746              |
| 2-Undecenal                      | 16.7473        | 0.703              |
| cis-4-Decenal                    | 11.369         | 0.643              |
| Hexadecanal                      | 23.9233        | 0.626              |
| Tridecanal                       | 18.2703        | 0.583              |
| 1,4-Hexadienal, (E,E)-           | 1.5929         | 0.489              |
| 13-Methyltetradecanal            | 22.142         | 0.476              |
| Benzaldehyde                     | 6.749          | 0.304              |
| 7-Octenal, 3,7-dimethyl-         | 14.745         | 0.236              |
| 2-Octenal, (E)-                  | 8.0924         | 0.187              |
| E-14-Hexadecenal                 | 21.717         | 0.162              |
| 2-Nonenal, (E)-                  | 10.5641        | 0.120              |
| Pentanal                         | 1.2641         | 0.103              |
| 2,6-Nonadienal, (E,Z)-           | 10.3965        | 0.076              |
| Benaldehyde, 2,4-dimethyl-        | 10.147         | 0.039              |
| Methional                        | 4.5646         | 0.029              |
| 1-Pentanol, 5-(methylenecyclopropyl)- | 14.1905      | 0.027              |
| 2-Pentenal, (Z)-                 | 1.932          | 0.004              |
| Butanal                          | 1.0876         | 0.004              |
| 2-Heptenal, (E)-                 | 24.6937        | 0.0003             |

**Alcohols**

| Compound                          | Retention Time | Relative Intensity |
|----------------------------------|----------------|--------------------|
| 1-Octen-3-ol                     | 6.3622         | 4.315              |
| 1-Octen-1-ol                     | 8.4543         | 1.672              |
| Heptanol                         | 6.1343         | 1.065              |
| Octanol                          | 8.5287         | 0.929              |
| Z-10-Pentadecen-1-ol             | 12.549         | 0.528              |
| 2-Nonyn-1-ol                     | 13.104         | 0.353              |
| Nonanol                          | 10.9319        | 0.312              |
| 2,4-Decadien-1-ol                | 7.809          | 0.119              |
| 5-Octen-2-yn-4-ol                | 15.389         | 0.109              |
| Pentanol, 5-(methylene cyclopropyl)- | 13.459        | 0.093              |
| E-2-Octadecadecen-1-ol           | 25.649         | 0.071              |
| 1-Pentanol                       | 2.283          | 0.042              |
| Hexanol                          | 3.8887         | 0.033              |
| Undecyn-1-ol                     | 12.927         | 0.027              |
| Pent-1-ol, (Z)-                  | 2.3192         | 0.010              |
| Pent-3-ol                        | 20.8861        | 0.001              |

**Ketones**

| Compound                          | Retention Time | Relative Intensity |
|----------------------------------|----------------|--------------------|
| Decanone                         | 13.6935        | 3.684              |
| Heptanone, 6-methyl-              | 6.3757         | 2.334              |
| Heptanone                         | 8.9342         | 0.584              |
| Heptanone, 6-methyl-              | 16.0912        | 0.350              |
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 [6]. According to [26], aroma description range of fresh Patin catfish are fresh, neutral and has species specific aroma and for the steamed one, its aroma description range are a bit fishy, fresh, neutral and species specific aroma. Taste descriptions of steamed Patin catfish are bit umami, slightly sweet and juicy.

Various volatile compounds that have been detected on both fish species were derived from sample's components, mainly from proteins and lipid content, so that the wide variety of quantities and volatile compounds are related to variation of chemical compounds contained in the samples. Most of those volatile compounds which contribute to commodities aroma were derived from the results of enzymatic reactions, microorganism activities, lipid auto oxidation, resulting substance from various thermal involved reactions and environmental impacts [7]. More varieties of volatile compounds than fresh samples have been detected and identified in processed fishery samples and their types and composition would depend on the sample type, chemical composition and processing methods. Several papers have described these in their research, among them were [14], [15], [17], [18], [22], [27].

In general, it can be assumed that processed fish possessed a higher number of flavour components compared to those of raw fish. One of the reasons is that the further increasing of such components was a result of biochemical pathways of protein and lipid of fish. The other reason may be the concentration of such flavour components was found to be much higher in volatile component analysis due to the reduced moisture content during processing which resulted in a higher concentration of flavour components in the final product. According to [14], flavour components detected in dried horse mackerel were formed probably as a result of oxidation of lipid as well as enzymic hydrolysis of the original components of fish such as lipid and protein. Thermal condition may accelerate retro-aldol degradation of unsaturated aldehydes which lead to altered flavour in these products. Other elements that could affect the numbers and types of compounds discovered in this volatile compound analysis were extraction method, type of samples and GC/MS column and its running parameters [17].

As mentioned above, most of the detected compound group originates from and could be categorized into several groups which were hydrocarbons, aldehydes, alcohols, ketones and their derivatives. Volatile compounds that came from hydrocarbon groups could derive from decarboxylation reaction and the splitting process of fatty acid’s carbon chains, a secondary reaction from carotenoid (if present) and unsaturated fatty acids thermal oxidations [4], [5], [10]. Aldehydes group compounds detected could derive from fatty acid carbon’s double bonds oxidation whether they were saturated or unsaturated [5], [8], [10], [22], [42], [43]. Alcohols, aldehydes and ketones groups volatiles compounds detected, were also could formed as a result of lipid and fatty acid oxidations and
amino acid degradation that occurred during processing [44], [45], [46]. Almost all reaction that could generate or produce volatile compounds would involve saturating and unsaturated fatty acids, which in general were abundantly contained in most of fishery commodities. The sensory differences could be expected among species depending on the lipid content since most of the volatile compounds in fish are derived from the oxidative breakdown of unsaturated fatty acids [29].

Some of the hydrocarbons, aldehydes, alcohols and ketone groups which detected in samples such as limonene, naphthalene, hexadecane, nonadecane, tridecane, dodecane, 1-nonadecene, 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 [10], wild and cultured sea bream [7] and raw black bream [22]. Ketone compound 2,3-pentanedione were known detected in fresh sardine and has a caramel like flavour characteristic. Aldehydes such as hexanal was detected in all the samples and known to have green-like flavour characteristic, methional that was detected in fresh patin and fresh and steamed Spanish mackerel has its almond/fruity/creamy/nutty aroma. Ketones group detected in samples are known to contribute to the sweet aroma of many crustaceans [6].

4. Conclusion

The moisture, ash, protein and lipid content analyses results from fresh and steamed samples of Patin catfish and Spanish mackerel were relatively differ. These measurement results are basically depending on the commodity type, initial chemical composition of the samples, feed, environmental factors and processing technique. Moisture loss during heating process will affect other contents measured. Lower moisture content in samples would result in higher numbers of other contents measurement. Amino acid analysis showed that both species steamed fish samples possess a relatively higher level of amino acids if compared to the fresh one. This increasement could be affected by proteolytic reaction that occurred during steaming and time length of the heat treatment. The highest amount of amino acids detected in fresh and steamed samples of both species is glutamic acid which imparts product’s umami taste. Volatile compound analysis showed that steamed Patin catfish and Spanish mackerel samples has higher quantities of volatile compounds compared to the compounds that were identified from fresh samples and most of the detected compound group could be categorized into hydrocarbons, aldehydes, alcohols, ketones and others (esters, nitrogenous compounds, furan). As much as 29 and 59 volatile compounds were detected from fresh and steamed Patin catfish samples, respectively, and 37 and 102 volatile compounds were detected from fresh and steamed Spanish mackerel respectively. It can be assumed from these results that volatile compounds found in marine water fish are higher in quantity compared to freshwater fish. The most abundant compound groups in both samples are aliphatic, cyclic and aromatic hydrocarbons which derive from decarboxylation reaction and the splitting process of fatty acid’s carbon chains, a secondary reaction from thermal oxidations of unsaturated fatty acids.
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