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Characterization of flavor-related compounds and sensory profiles of four fermented fish products prepared from silver rasbora (*Rasbora argyrotaenia*) and anchovy (*Stolephorus* sp.)

Rachmawati, S.H., Widiastuti, I., Ridhowati, S., Umami, A., Vandiwinata, I. and *Lestari, S.D.

Fisheries Product Technology Department, Sriwijaya University, Ogan Ilir, 30662, Indonesia

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Abstract

Fermented fish products are grouped into salt and salt-carbohydrate types based on the fermentation substrate. A series of biochemical reactions on the substrate affects the flavor characteristics of the resulting products. This study compared the amino acids, fatty acids, and volatile compound profiles of four fermented fish products: bekasam, rusip, fish paste, and fish sauce made from silver rasbora and anchovy. The quantitative descriptive analysis (QDA) was carried out to determine the sensory profiles of those products. The results indicated that rusip and bekasam, which belong to salt-carbohydrate fermented fish, were characterized as high in arginine, stearic, capric, and oleic acid, with ketones, lactones, and fatty acid derivative compounds dominating their flavor profiles. The flavor profile of fish paste was characterized by high concentrations of aldehydes such as 3-methylbutanal and hexanal, whereas that of fish sauce was characterized by large concentrations of pyrazine and aromatic compounds. Based on the findings of the QDA, no significant difference was observed within the sensory variables of fish sauce and fish paste prepared from anchovy and silver rasbora, indicating that both fish are equally usable as raw materials. Whereas different saltiness was identified in both rusip and bekasam prepared from anchovy and rasbora. The results of PCA on QDA data separated fermented fish type 1 from the type 2. Fermented fish type 1 was more correlated with umami and sweet taste, while type 2 was correlated with fishy, salty, sour, and astringent taste. The result is useful as a basis for further product development and quality control.

1. Introduction

Indonesia is rich in fermented food diversity. Those foods have existed for many years and contributed to not only preserving the food but also forming an integral part of the diet (Surono, 2016). The interest of consumers in fermented fish products is primarily due to the specific flavor generated, which can induce appetite (Giyatmi and Irianto, 2017). Traditional fermented fish products are divided into three classes based on the substrate and source of the enzymes utilized during fermentation (Saisithi, 1994). The products in the first category are fermented by a bacterial enzyme that is already present in the fish and salt mixture, without the addition of carbohydrates. The second group consists of products in which carbohydrates are introduced to the fish and salt mixture and utilized during the fermentation process. In the last group, fermentation was accomplished with the aid of tissue enzymes and starter culture.

Bekasam and rusip are traditional fermented fish products that have been consumed in some areas in Sumatera and Bangka Belitung. Both products are categorized under the salt-carbohydrate type, although the type of carbohydrate added is different in each. Cooked rice, rice bran, roasted rice, and palm sugar are types of carbohydrates that are commonly added during preparation of some fermented fish products. The addition of carbohydrates such as rice or brown sugar stimulates the growth of carbohydrate-metabolizing microbes, which play an important role in the final sensory attributes of the products. On the other hand, fish sauce (kecap ikan) and fish paste (terasi ikan) are prepared by mixing fish with salt in a certain ratio, which therefore categorizes them as type I fermented fish. The fermentation times of both products are different, in that the fish paste fermentation takes a shorter time than the sauce. Although fish sauce and paste have been widely popular and similar products are found in many countries with various names, bekasam and rusip are more locally produced and consumed.

*Corresponding author.
Email: *shantidwita_thi@unsri.ac.id*

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In South Sumatera, bekasam is commonly prepared from small freshwater fish, including snakeskin gourami, climbing perch, and silver rasbora. However, larger fish such as carp, tilapia and pangasius can also be used as raw materials. Rusip is generally made using anchovies and its liquid appearance makes it classified as fish sauce. However, it has distinct properties compared to other types of fish sauce such as budu (Malaysia) and nam pla (Thai), or bakasang (Moluccas) which is made only with a mixture of fish and salt. Palm sugar is added to the process of making rusip and the fermentation time is shorter so that the fish is still not completely digested.

Lactic acid bacteria have been reported to be a member of the fermented fish type 2 microbial community, such as in Thai plaa-som which prepared from fish, salt, palm syrup and occasionally cooked rice (Kopermsub and Yuncharald, 2010), bekasam (Desniar *et al*., 2013), and rusip (Lestari *et al*., 2021) whereas the microbial community of type 1 fermented fish is dominated by halophilic and halotolerant microbes (Das *et al*., 2020). In fermented products, the microbiota has a role in the formation of sensory qualities, such as taste, aroma, and texture. The existence of this microbiota generally comes from raw materials. The interaction of microbes with the substrate (type of fish) and the composition of it will affect the final characteristics of the fermented product produced.

The use of different raw materials is assumed to result in a fermented fish product with different sensory properties. Marine fish and freshwater fish tend to have different microbial communities, which dominate their intestine, gills, and skin. The nutritional composition, especially protein and lipids, and presence of flavor precursor compounds might be different from one type of fish to another. The flavor of marine fish is different from that of freshwater fish, and the variation can be attributable to the content of carbonyls and alcohols. Bromophenols are abundant in some marine fish species while absent in freshwater ones. These compounds are responsible for the sea salt, brine, shellfish, iodine, and phenol-like flavor and contribute to the differences between marine and freshwater fish (Lindsay, 1994). Although the variety of flavors produced by fermented fish products are important factors in satisfying consumers' tastes, the comparison of fermented fish produced from marine and freshwater in terms of volatiles and sensory attributes has been scarcely studied.

The quantitative descriptive analysis (QDA) method is a product-based sensory profile identification method using a trained panel approach. In QDA, the panelists quantify the intensity of specific attributes of a product by using the line scale. Although the length of the line may vary, the 15 cm scale is often used for its better discriminative capacity and the chance given to the panelists to distribute their ratings more evenly across this scale length (Gomide *et al*., 2021). The results of QDA are amenable to multivariate data analysis (Chapman *et al*., 2000). By using principal component analysis (PCA), the identity of the principal component contributing to the attribute differences between fermented fish products prepared from freshwater fish and marine fish can be determined. The profiles of fermented fish made of different raw materials can be compared and used as a basis for further product development by noting the attributes important to consumers. Such data will also be beneficial as a reference for process modification or alteration of product characteristics to improve consumers' acceptance.

2. Materials and methods

2.1 Sample preparation

Bekasam was made by mixing gutted fish with 15% (w/w) of salt and 15% (w/w) of cooked rice. The mixture was placed in containers with airtight lids and fermented for seven days at room temperature. To prepare rusip, the fish was rinsed with clean water and combined with 25% (w/w) salt. The mixture was added with liquid palm sugar at a ratio of 1:10 (v/w) to the fish's weight. After thorough mixing, the mixture was transferred to containers and fermented for seven days at room temperature. Fish sauce (kecap ikan) was prepared by mixing thoroughly portion of ungutted fish with 25% salt (w/w). The mixture was placed in a 1 L glass jar, sealed, and left to ferment for approximately 1 year. To make fish paste (terasi ikan), fish was rinsed with water and half-dried under the sun. The half-dried fish was weighed, and 20% (w/w) salt was added before being crushed together until even and stored in tight containers for 18-24 hrs for initial fermentation. The mixture was finely pounded and dried in the sun for a day. Following the further pounding, the resulting paste-like substance was wrapped in banana leaves and fermented for another two weeks. The fermented fish were then used for sensory and flavour evaluation.

2.2 Quantitative descriptive analysis

QDA analysis was carried out by involving seven trained panelists who were the final-year students of the Fish Product Technology Department, Sriwijaya University, and had passed the pre-screening, selection, and training procedures as proposed by Meilgaard *et al*. (2007). During the pre-screening stage, twenty-five students who have passed a sensory analysis course were tested for the identification of basic tastes, which include sweet, salty, umami, and sour. The panel candidates were also tested for triangle testing. The criteria for passing the selection were meeting 100% of the basic taste detection and smell recognition requirements as well as responding correctly to 60% of the triangular discrimination test carried out. The panelists who passed the pre-screening stage entered the subsequent stage, where they did the attribute determination based on focus group discussion. The captured attributes were used in the process of training and strengthening the attributes of commercial bekasam and rusip samples. In this stage, panelists were also exposed to a line scale in which the strength of an attribute grows from left to right, with "weak" and "strong" phrases representing "weak" and "strong" stimulus intensity (Gomide *et al*., 2021). The task of the panelists were to provide a value to the scale that indicates the intensity of the characteristic being assessed. The training procedure starts with a series of tests to determine the consistency of potential panelists' responses to key characteristics in relation to the amount of taste and aroma concentration treatment in each attribute. Based on the findings of the serial test, seven panelists with a strong capacity to assess the strength of the taste and aroma were chosen to perform QDA.

2.3 Amino acid and fatty acid analyses

The amino acid analysis was conducted using HPLC with a fluorescence technique for detection. Prior to analysis, the samples were hydrolysed in 6 N HCl for 24 hrs at 110°C and the excess of solvent was removed using a rotary evaporator. The concentrated samples were diluted with 0.01 N HCl into 10 mL and used for amino acid analysis. The protein content of the samples was determined by Kjeldahl method whereas the total lipid was determined by Soxhlet extraction with hexane as solvent. Fatty acid analyses were carried out using GC -FID by injecting samples that have been converted to fatty acid methyl esters (FAME). Amino acids and fatty acids were identified by comparison of their retention times with their respective standards and their contents were calculated on a weight percentage basis.

2.4 Volatile component analysis

The isolation of volatile compounds was done using SPME technique following a method Kleekayai *et al*. (2016). Approximately 3 g of sample was transferred to a 22 mL headspace vial and sealed tightly with an aluminum cap with polytetrafluoroethylene/silicone liner (Supelco, PA, USA). The vial was heated at 50°C for 10 mins. Headspace volatile components were adsorbed for another 20 mins by a DVB/CAR/PDMS) SPME fiber (Supelco) and injected to gas chromatography (Agilent Technologies, USA). The desorption temperature of 250°C of for 20 mins was employed with normal oven incubation. The headspace volatile components were separated in a DB-WAX capillary column and identified using mass spectrometer (Agilent Technologies, USA). Electron ionization (EI) at 70 eV and a scan range of 40-350 AMU was used to get the mass spectra of volatile compounds. The compounds were identified by comparing mass spectra and retention durations from library databases (National Institute of Standards or NIST and Wiley 275).

2.5 Statistical analysis

The data obtained from QDA were analysed statistically by ANOVA, and the mean differences of each sensory attribute of the products made from different fish were evaluated to identify the significant variations using the Minitab 19.1 (Minitab LLC, USA). The data obtained from amino acid, fatty acid GC-MS and sensory analysis were subjected to multivariate data analysis performed using the MetaboAnalyst 5.0 (Canada). The principal component analysis of the QDA data was performed using XLSTAT 2019 software (AddinSoft, USA). Visualization of multivariate data was obtained from the Clustvis (Metsalu *et al*., 2015).

3. Results and discussion

3.1 Sensory analysis

It is well recognized that sensory quality of fermented fish can greatly affect the consumer's acceptance. Each product has specific sensory attributes and flavor which formed after series of enzymatic and non-enzymatic reactions that degradation protein, fat, and carbohydrates. Prior to QDA, eight attributes were described for bekasam samples based on the focus group discussion which included salty taste, umami taste, fishy taste, astringent taste, sour taste, bitter taste, fishy aroma, and acidic aroma. Whereas, for rusip samples, the attributes were salty taste, umami taste, fishy taste, sour taste, bitter taste, fishy aroma, acidic aroma and salty aroma. The result of sensory analysis is presented in Figure 1. The spider webs showed the dominant attribute of each product based on the species used as raw materials. The dominant attribute for fish sauce was salty taste followed by fishy aroma and fishy taste. The salty taste was correlated with the addition of salt during fish sauce preparation.

The predominant sensory characteristic of fish paste was its fishy odor, followed by its fishy flavor. When anchovies were employed as a raw material, the fishy odor and taste were amplified. On the other hand, the flavours of rasbora-based items were reported to be more astringent, salty, and umami. However, there was no statistically significant difference ($p>0.05$) in the sensory characteristics of fish sauce and fish paste, indicating that both raw materials may be used to manufacture fish

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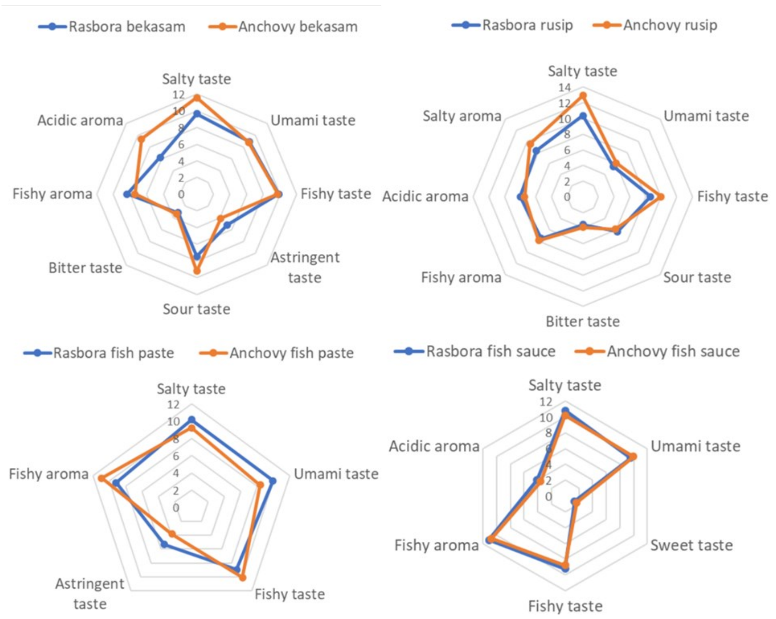

Figure 1. The sensory profiles of four fermented fish prepared from anchovy and silver rasbora. The position of individual attributes on a wheel around a center representing attribute intensity scales, with higher values radiating outward.

sauce and fish paste.

In rusip, there are more sensory characteristics detected by trained panelists. The acidic taste and aroma were recognized. These characteristics were caused by the bacteria' transformation of glucose from palm sugar into organic acids such as lactic acid. In addition to this, a very faint bitter taste was also observed. Certain forms of free amino acids, which are products of protein breakdown, contributed to the bitter flavor. The two most important characteristics of rusip were its salty and fishy flavor. The ANOVA revealed a significant difference ($p<0.05$) between the saltiness of anchovy and rasbora rusip, although no significant differences were found for other characteristics.

The predominant flavors of bekasam were salty, fishy and sour. The sourness of bekasam results from the fermentation of carbohydrates, and its intensity is substantially greater than that of rusip. This characteristic is associated with the metabolic process of acid-producing bacteria that transform carbohydrates into organic acid. ANOVA indicates that the aroma and taste of anchovies bekasam are substantially more acidic and saltier than those of rasbora ($p<0.05$).

The biplot build based on QDA data (Figure 2) shows that the fermented fish type 1 (denotated by letter FS and FP) forms a cluster on the left side of the principal component 1 (F1) while the fermented fish type 2, rusip and bekasam are clustered together on the right side of F1. The principal component 1 explained 29.18% of the variability of sensory attributes of 4 different fermented fish products studied. From the same plot, most of panellists perceived fish sauce and fish paste for their sweet, umami taste and fishy aroma while bekasam and rusip were correlated with astringent, sour, fishy, bitter, and salty taste with acidic and salty aroma. From the same plots, it can also be concluded that the distribution pattern of four types of fermented fish made from 2 different species were more affected by the type of products than the type of fish.

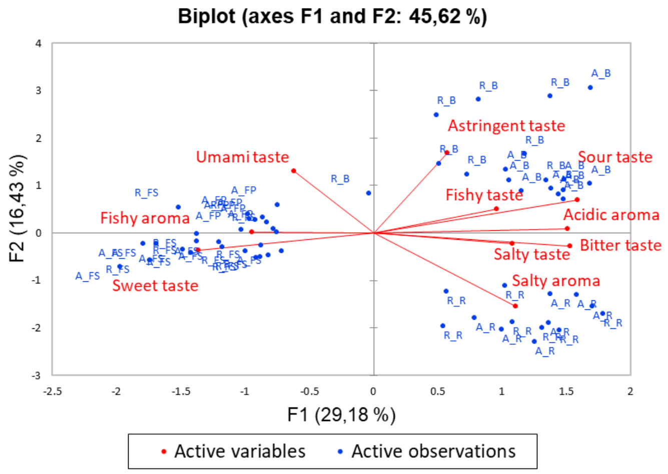

Figure 2. PCA biplots based on sensory profiles of four fermented fish prepared from anchovy and silver rasbora.

3.2 Amino acid and fatty acid profiles

Glutamate was the most dominant amino acid found in all types of fermented fish, followed by aspartic acid and lysine (Table 1). The amino acids, such as glutamic acid, aspartic acid, alanine, and glycine are directly responsible for flavour and taste and can be precursors of aromatic components. Changes of amino acids during fish storage are initiated by muscle autolysis and at the later stages, changes are facilitated by the growth of microorganisms (Ozden, 2005). The microbial metabolism of protein results on amino acids and peptides which give the fermented fish their characteristic taste and flavour.

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Table 1. Amino acids content of four fermented fish prepared from anchovy and silver rasbora.

Type	1				2			
Product	Fish paste		Fish sauce		Bekasam		Rusip	
Species	Rasbora	Anchovy	Rasbora	Anchovy	Rasbora	Anchovy	Rasbora	Anchovy
Aspartic acid	1.48	1.79	1.06	1.12	1.12	1.56	1.14	1>27
Threonine	0.81	0.9	0.58	0.63	0.52	0.78	0.56	0.63
Serine	0.63	0.64	0.31	0.32	0.47	0.61	0.5	0.5
Glutamate	2.61	2.78	2.9	3.23	2.48	3.,55	2.37	2.63
Glysine	1.09	1.14	0.84	0.67	1.05	0.89	1	0.81
Alanine	0.98	1.15	0.8	0.83	0.86	1.,2	0.85	0.86
Valine	0.921	1.07	0.69	0.8	0.59	0.88	0.58	0.66
Methionine	0.53	0.63	0.31	0.33	0.37	0.51	0.13	0.38
Isoleucine	0.76	0.87	0.73	0.95	0.58	0.88	0.58	0.67
Leucine	1.28	1.36	1.21	1.31	0.95	1.43	0.95	1.09
Tyrosine	0.54	0.63	0.43	0.49	0.32	0.43	0.2	0.26
Phenylalanine	0.76	0.83	0.68	0.73	0.56	0.76	0.52	0.59
Histidine	0.53	0.61	0.36	0.35	0.3	0.44	0.37	0.37
Lysine	1.32	1.51	1.05	1.14	0.99	1.75	1.12	1.22
Arginine	1.07	1.21	0.25	0.18	1.5	1.49	1.67	1.45

The comparison of amino acid profiles between products are shown in Figure 3. The heatmap indicates that the anchovy paste (AP) had the highest content of amino acids especially histidine, aspartic acid, valine, glycine, tyrosine, and methionine. Whereas anchovy bekasam had the highest glutamic acid, lysine, alanine, and leucine. Indonesian fish paste which is known as terasi in local language is famous for its strong odor. The solid-state fermentation combined with series of sun drying and pounding may have resulted in high volatile constituent of the finished product (Al Jedah and Ali, 1999). This statement is in line with the results of flavor analysis (Figure 5) in which fish paste had wider variety of volatile compounds with high concentration. The relatively low moisture content as an effect of sun exposure during terasi preparation is attributable for the high amino acid content of this paste product.

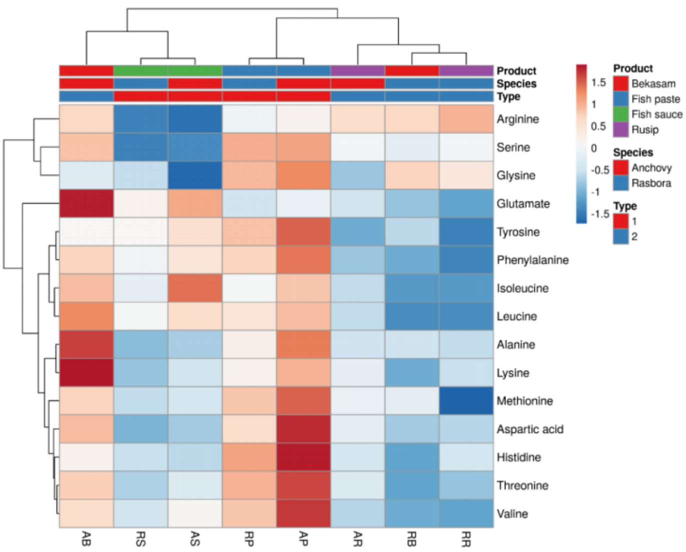

Figure 3. Heatmap of amino acid profiles of four fermented fish prepared from anchovy and silver rasbora.

The results of fatty acid analysis of different fermented fish products are simultaneously compared and presented as heatmap (Figure 4) to highlight the content differences between samples. Bekasam prepared from anchovy had the highest EPA, DHA, arachidonic and nervonic acid whereas the one prepared from silver rasbora was higher in medium chain fatty acids. In general, products prepared from anchovy had higher fatty acids content than those of rasbora.

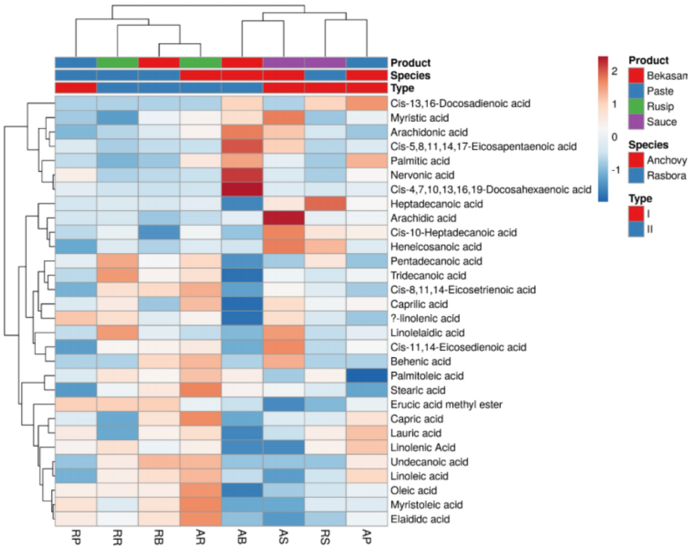

Figure 4. Heatmap of fatty acid profiles of four fermented fish prepared from anchovy and silver rasbora.

3.3 Assessment of volatile compounds

The relative contents of the volatile components of four fermented fish products prepared from anchovy and silver rasbora are depicted on Figure 5. In general, the development of distinctive flavor and aroma of fermented fish occurs as fermentation progresses and combination of compounds' notes contributes to the odor of fermented fish that eventually smelt by consumers and plays rote in appetite-inducing. Those compounds are derived from the hydrolysis of protein and oxidation of

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lipid brought about by combination of autolytic and microbial activity (Beddows *et al*., 1980; Saisithi, 1994). Alcohols, alkanes, acids, esters, aldehydes, hydrocarbons, lactones, and ketones are considered as essential contributors to the characteristic flavor of fermented fish (Zeng *et al*., 2017; Sharma *et al*., 2020).

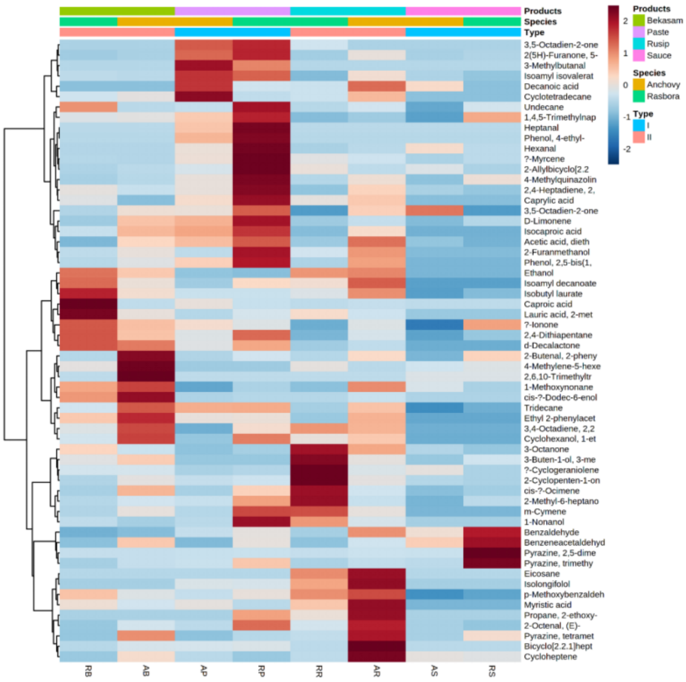

Figure 5. Heatmap of volatile compounds profiles of four fermented fish prepared from anchovy and silver rasbora.

Based on the GC-MS analysis, the dominant volatile components of rasbora fish sauce as presented in Figure 5 were primarily comprised of aldehydes (benzaldehyde, benzenacetaldehyde) and aromatic compounds such as pyrazine. Fukami *et al*. (2002) reported four odour-contributing compounds identified in fish sauce; 2-methylpropanal and 2-methylbutanal which belong to aldehydes; and 2-ethylpyridine and dimethyl trisulfide which contribute to the grassy green and cooked aroma, respectively (Parker, 2015). Fish paste has more volatile compounds with present in high concentration compared to other type of fermented fish. The volatile compounds of fish paste were dominated by 3,5-octadien-2-one, 2,4 heptadiene, 3-methylbutanal, heptanal, hexanal, 4-ethyl phenol, 2-myrcene, 1-nonanol and D limonene which belong to aldehyde, ketone, alcohol and aromatic groups. One of the dominant compounds, 3,5-octadien-2-one is known to have a fruity, green, grassy odor and responsible for chestnut-like aroma quality of green teas (Zhu *et al*., 2018). The presence of this compound in fish paste may be attributable to the banana leaves used to wrap the paste during fermentation.

Rasbora rusip was high in ketones (3 octanone, 2 cyclopentene 1 one, 2 methyl 6 heptanol, 3-buten-1-ol, 3 -methyl-) while the anchovy rusip was high in acids, alkanes, and fatty acid derivatives such as eicosane and myristic acid. The product of aerobic fermentation of fish with an ample supply of oxygen tend to have a richer profile of volatile fatty acids which often referred as cheesy flavor. Acid producing bacteria are known to be responsible for the formation of acetic, propionic, isobutyric and isovaleric acids fractions which contribute to the flavor of Thai fish sauce (Al Jedah and Ali, 1999). Bekasam was generally contain high d-decalactone compared to other type of fermented fish. Ethyl 2 phenylacetate and 1 methoxynonane. The difference between 2 species used in bekasam was explained by high caproic acid and lauric acid, 2-methylbutyl ester in rasbora bekasam whereas, high 2 phenyl 2 butenal, 4-methylene-5-hexenal, 2,6,10-trimethyltridecane, cis-γ-dodec-6-enolactone in anchovy bekasam. Ethanol was consistently noticeable in salt-carbohydrate fermented fish observed in this study, however, this compound was quite low in fish sauce and fish paste. Due to their high detection threshold values, the volatile compound belong to alcohol groups may not have a significant impact on the odor. On the other hand, aldehydes can contribute to the unique aroma of fermented fish because of their low thresholds (Zeng *et al*., 2017).

The Figure 6 shows the clustering of fermented fish products based on their volatile compounds profile. This data visualization reinforces the findings of QDA data and confirms that the distribution pattern of four types of fermented fish made from 2 different species were more affected by the type of products than the type of fish.

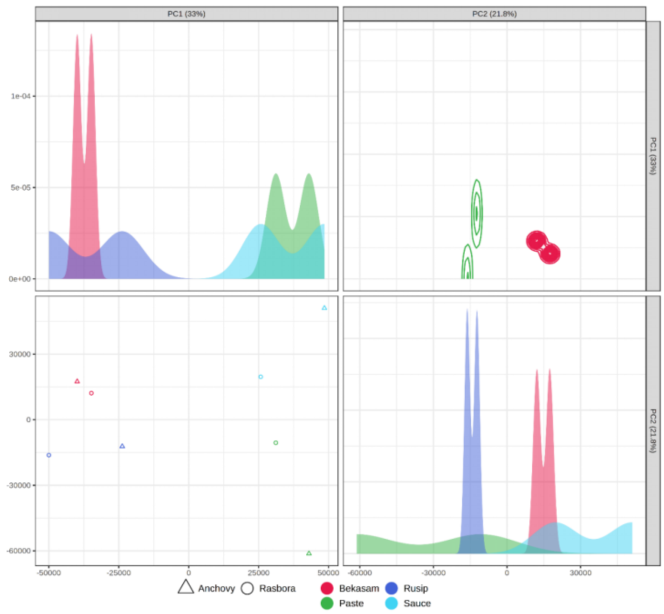

Figure 6. Principal component analysis built from volatile profiles of four fermented fish prepared from anchovy and silver rasbora.

4. Conclusion

The biotransformation of macromolecules that takes place during fermentation is regulated by the chemical properties and composition of the microbiota present in the raw materials and the fermentation environment. Different amino acids, fatty acids, volatile compounds and sensory attributes were examined in four fermented fish belong to different type and prepared form fresh water and marine water fish. The distribution pattern of four varieties of fermented fish made from two distinct species was influenced more the type of product than by the species of fish. Based on the principal component analysis of the QDA data, type 1 fermented fish, represented by fish sauce and fish paste, was correlated to fishy aroma, umami, and sweet taste, whereas type 2 fermented fish was correlated to fishy, salty, bitter, sour, and astringent taste with salty and acidic aroma.

Conflict of interest

The authors declare no conflict of interest.

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