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Physicochemical properties, urocanic acid and biogenic amine contents of black pomfret (*Parastromateus niger*) as affected by fermentation times

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Abstract

Fermented freshwater and marine products are popular in Malaysia and among these is *ikan pekasam* (fermented fish). It is typically made from freshwater fish with ground-roasted rice as the main source of carbohydrate during fermentation. In this study, rather than freshwater fish, black pomfret (*Parastromateus niger*), a marine fish, was used to produce *ikan pekasam.* Black pomfret was mixed with 3 kg of ground roasted rice and 10% (v/w of fish) water and allowed to ferment for 2 and 5 weeks at 25 – 30°C. Then, fermented black pomfret was analysed for its physicochemical properties, compositions of biogenic amine, urocanic acid (UCA) and organic acid, as well as sensory acceptability. The pH and titratable acidity significantly ($p \leq 0.05$) decreased and increased, respectively, after 5 weeks of fermentation due to the increase in organic acids, mainly lactic acid. Histamine, putrescine, cadaverine and 2-phenylethylamine increased rapidly after 5 weeks of fermentation but histamine was within the recommended level (50 mg/kg; US Food and Drug Administration (USFDA)). The strong correlation between *trans*- and *cis*-urocanic acid indicated that photoisomerization of *trans*-UCA into *cis*-UCA occurred. Overall, principal component analysis (PCA) revealed that the two-week fermented black pomfret was more acceptable to the consumer panellists than the five-week due to its acceptable aroma, saltiness, sourness and texture. Notably, this two-week sample had significantly ($p \leq 0.05$) lower biogenic amine and UCA contents; suggesting a safer and good quality of *ikan pekasam*.

1. Introduction

Ikan pekasam (Malaysian traditional fermented fish) is typically produced using freshwater fish because of its earthy flavour and firm texture (Huda, 2012). However, freshwater fish are less preferred by some consumers because of the possible presence of parasites (Iyaji and Eyo, 2008; Theerawoot, 2008; Ljubojevic *et al*., 2015). Marine fish have a fishy and ammoniac odour (Tanasupawat and Visessanguan, 2014), high level of nutrients (Rosli *et al*., 2012; Reksten *et al*., 2019) and an insignificant parasite problem (Riebroy *et al*., 2007; Poulin, 2016), hence, can be used in the production of *ikan pekasam*. However, depending on age and gender, marine fish provide less of the recommended daily mineral and trace elements (Mn and Cu intake 4–9.9, 1.2–2.3 and 0.3–0.9 mg/day, respectively) intake than freshwater fish (Rahman *et al*., 2020). Nonetheless, the quality of produced *ikan pekasam* from marine fish species found in Malaysia has not yet been reported.

Ezzat *et al*. (2015) reported no significant differences in the content of histamine and many other biogenic amines, while *trans*- and *cis*-urocanic acid (UCA) and total UCA content were significantly different in fifteen commercial *ikan pekasam* samples produced from black tilapia and Javanese carp. Black pomfret (*Parastromateus niger*) or *bawal hitam*, a marine fish, is popular in Malaysia and available all year round. Black pomfret is a rich source of amino acids that make it a potential antioxidant (Nazeer and Sampath Kumar, 2011). It has a flat shape suitable for fermentation, with fermented black pomfret exclusively served as a gourmet dish. It is not a scombroid fish, like tuna and mackerel, that has been implicated in histamine poisoning (Zare *et al*., 2013; Tortorella *et al*., 2014; El-

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Ghareeb *et al*., 2021), hence, it could be used to produce good quality *ikan pekasam*. The present study prepared *ikan pekasam* from black pomfret and evaluated the physicochemical properties, including pH, titratable acidity and salt content, and the biogenic amine, UCA, and organic acid content. Sensory analysis of black pomfret after 2 and 5 weeks of fermentation was also performed. Then, the correlation between sensory acceptability, biogenic amine and UCA contents was determined using principal component analysis (PCA).

2. Materials and methods

2.1 Materials

Fresh black pomfret (each weighing 150±10 g, 16±3 cm in length) and coarse salt were purchased fresh from a wholesale market, in Selangor, Malaysia. The fish were kept in a plastic box on crushed ice and transported immediately to the laboratory for further processing. Ground roasted rice for fermentation was prepared by roasting uncooked white rice over a slow fire for 30 mins until it turned brown, followed by grinding and sieving through a metal sieve (mesh size 11.2 mm) (BS Ever, Anping Blue-Star Co. Ltd, China).

Histamine dihydrochloride (97%), tyramine hydrochloride (99%), putrescine dihydrochloride (99%), cadaverine dihydrochloride (97%), spermidine trihydrochloride (98%), spermine tetrahydrochloride (97%), tryptamine hydrochloride (98%), 2-phenylethylamine hydrochloride (99.5%), formic acid (95%), L-pyroglutamic acid (99%), malic acid (99%), succinic acid (99%), *trans*- and *cis*-UCA, and analytical grade benzoyl chloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Fumaric acid (99%) and acetic acid (100%), ammonium acetate, sodium salt of 1-octane sulphonic acid, HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Double-distilled deionized water was produced from the Milli-Q system (Millipore, Bedford, MA, USA). The citric acid (99%), tartaric acid (99%) and oxalic acid (100%) were purchased from Fisher Scientific (Rochester, NY, USA) while lactic acid (85%) was obtained from JT. Baker (Central Valley, USA).

2.2 Sample preparation

The black pomfret was scaled, degutted, then cleaned under running tap water and stored at 4°C. For fermentation, 3 kg of degutted and cleaned black pomfret were first mixed well with 20% of coarse salt (w/w) and placed in a lidded plastic container (19.5 cm diameter × 23.0 cm deep). After 3 days at ambient temperature (25−30°C), the fish were rinsed under running tap water and returned to the container, then mixed with 3 kg of ground roasted rice and 10% (v/w of fish) water. The fish were allowed to ferment for 0 (Control), 2 and 5 weeks at ambient temperature (Figure 1) with 3 replications. The addition of ground roasted rice provided a source of fermentable carbohydrates to support lactic acid bacteria (LAB) growth, as well as the development of colour characteristics and mask the fishy odour of the final fermented product (Huda, 2012; Mahyudin *et al*., 2015).

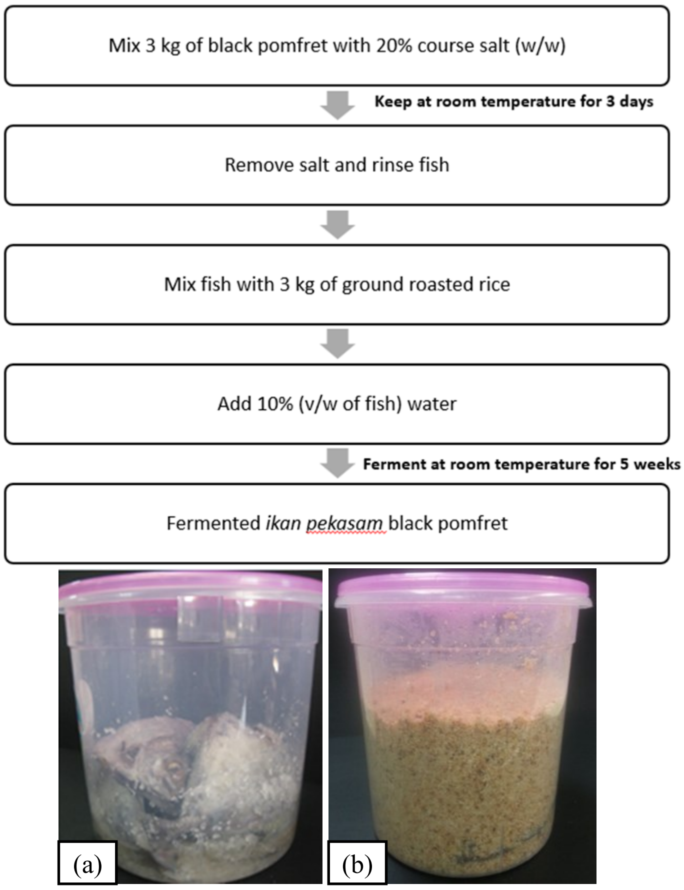

Figure 1. Production of *ikan pekasam* black pomfret: (a) salting and (b) fermentation process.

The *ikan pekasam* samples from each batch were manually cleaned to remove external ground roasted rice, bones, and heads, then the muscles and skin were homogenized for 3 mins using a blender (Warring 32BL79, USA), transferred to a separate plastic bottle, sealed, and stored at -20°C until further analysis.

2.3 Determination of salt content, titratable acidity and pH

Titratable acidity (% lactic acid) and pH were determined based on the methods of Association of Official Analytical Chemists (AOAC) (2019). A salt meter (Atago ES-421, 0-28% Salinity, Tokyo, Japan) was applied to determine the salt content as % sodium chloride (NaCl) (Zaman *et al*., 2011).

2.4 Evaluation of biogenic amine content and composition

HPLC analysis, biogenic amines extraction and

https://doi.org/10.26656/fr.2017.8(2).275

derivatization from representative samples were accomplished according to the method of Ezzat *et al*. (2015). A series of working solutions of mixed standards were prepared by diluting stock solutions with 0.1 M HCl to obtain a range of concentrations between 0.001 to 0.01 mg/mL for each biogenic amine. The solutions were then derivatized like the samples for HPLC analysis. A standard calibration curve for each biogenic amine was generated by plotting the peak area versus the corresponding concentrations in the final solution injected.

2.5 Evaluation of trans- and cis-UCA composition and content

The content of *trans*- and *cis*-UCA was determined according to the procedure reported by Zare *et al*. (2012) using the same HPLC device, column and software as described for biogenic amine analysis. A solution of 25 mM citrate buffer (pH 3.2) and acetonitrile in a ratio of 88:12 including sodium octane sulfonate (3 mM) was applied as mobile phase. The temperature of the column was set at 30°C and peaks were detected at 267 nm.

The samples were prepared by adding 2.5 g of *ikan pekasam* sample to a 40 mL centrifuge tube containing 2.5 mL of 0.05 M HCl, vortexing and incubation in a water bath for 10 mins at 80°C. The tubes were then sonicated (Ultrasonic cleaner bath, Elmasonic S 30H, Singen, Germany) at 40 kHz for 30 mins at ambient temperature and centrifuged at 10,000×*g* and 10°C for 20 mins. The protein was precipitated with the addition of 600 µL of 10% trisodium citrate solution and 1.0 mL of 10% citric acid solution in a sequence to 1.0 mL of the supernatant obtained above and vortexed for 1 min. Finally, the tubes were placed at ambient temperature for 10 mins, then centrifuged at 8000×*g* for 10 mins.

The injection volume and flow rate were 20 µL and 1.0 mL/min, respectively and the samples were filtered using a syringe membrane filter with a pore size of 0.22 µm before injection. *Trans*- (99% pure) and *cis*-UCA (≥ 98% pure) were dissolved in 0.05 M HCl to prepare stock standard solutions (1.0 mg/mL) and a series of working solutions ranging from 0.001 to 0.01 mg/mL were made by dilution in 0.05 M HCl. Standard calibration curves were drawn based on peak area versus the corresponding concentration of injected standard solutions.

2.6 The content and composition of organic acids

The content and composition of organic acids were analysed according to the method of Ezzat *et al*. (2021). Identification of organic acids was based on the comparison of the retention times and known standards. A series of standard solutions of organic acid ranging between 0.2 mg/mL to 1 mg/mL were prepared in deionized water and filtered through a nylon membrane filter (0.22 µm) before injection. The organic acids content was calculated from the calibration graphs plotted based on the peak areas versus the injected concentrations.

2.7 Sensory evaluation

Whole fermented fish including ground roasted rice with an approximate weight of 150 ± 10 g were fried in a deep fryer (Philips HD 6158/55, 300 mm × 265 mm, Kuala Lumpur, Malaysia) containing 1.2 L refined, bleached and deodorised (RBD) palm olein (Cap Buruh, Lam Soon Edible Oils, Malaysia) at an initial temperature of 170±2°C for 6 mins. The oil was replaced after every two frying cycles. Once fried, the residual oil was removed by placing samples on a piece of dry, clean tissue paper for 5 mins. The samples were then cut into 20 g pieces, placed in a small, covered plastic plate and stored at 70°C until the analysis, approximately 5 mins.

2.7.1 Evaluation of ikan pekasam black pomfret using quantitative descriptive analysis

Seven trained panellists (5 females and 2 males) who passed all four selection processes participated in the four training sessions which were conducted in the Discussion Room of the Sensory Laboratory to familiarize themselves with QDA. The *ikan pekasam* black pomfret samples that had been fermented for 2 and 5 weeks and fried were served with a carrier (a bowl of hot white rice), numbered by three-digit figures and presented to the trained panellists randomly (Stone *et al*., 2020). First, the appearance and odour of the fried *ikan pekasam* samples were evaluated by the trained panellists using the QDA questionnaire. Then, they were required to taste the samples before evaluating the texture and aftertaste (Table 1). A glass of filtered water and a plain cracker were provided between samples.

2.7.2 Sensory evaluation using the 9-point hedonic scale

The sensory evaluation of fried i*kan pekasam* black pomfret was conducted using 50 untrained consumer panellists using a 9-point hedonic scale. Two different samples (20 g) of fried *ikan pekasam* that had been fermented for 2 and 5 weeks were evaluated simultaneously, together with a carrier (hot white rice). Drinking water and plain crackers were provided to the consumer panellists, who were asked to rinse their mouths after each sample. They were asked to assess the aroma, appearance, hardness, saltiness, sourness and overall acceptability using a score ranging from 1 (Dislike extremely), 2 (Dislike very much), 3 (Dislike moderately), 4 (Dislike slightly), 5 (Neither like nor

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Table 1. Definitions of terminology for the attributes of *ikan pekasam* black pomfret in the QDA (Azman, 2014).

Attributes	Description
A. Appearance	
Brown colour	The strength or intensity of brown colour from light to dark
Roughness	A rough surface marked by irregularities, protrusions, grains, or bumps
B. Odour	
Rice grain	The aroma associated with heated rice grains (the rice was heated until there was caramelization of some carbohydrates such as starch)
Fishy	Suggestive of fish odour
Sourness	The aroma stimulated by acid or rancid by fermentation
C. Flavour	
Sourness	an acid or rancid taste
Saltiness	a salty taste
Bitterness	unpleasantly sharp taste
Fishy	suggestive fish taste
Fermented flavour	volatile flavour compound naturally created in fermentation
Roasted rice flavour	flavour derived from heated rice grains (the rice was heated until caramelization occurred of some carbohydrates such as starch)
D. Texture	
Cohesiveness (fish flesh)	mass cohesiveness of semisolid sample
Crispiness (fish skin)	sample breaks or fractures through involving noise and force
Oiliness texture	oily film presents on the oral surface
E. Aftertaste	
Tongue itchiness	itchy tongue/palate/throat after the product is swallowed

dislike), 6 (Like slightly), 7 (Like moderately), 8 (Like very much) to 9 (Like extremely) (Stone *et al*., 2020).

2.8 Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) and 95% confidence intervals ($p \leq 0.05$) between means were calculated using Tukey's multiple range test in Minitab V.19 (Minitab Inc., State College, Pennsylvania, USA). Pearson correlation and PCA were used to analyse the correlation between salt content, titratable acidity, pH, biogenic amine, UCA, organic acid, the intensity of sensory attributes and sensory acceptability of *ikan pekasam* black pomfret after 2 and 5 weeks of fermentation.

3. Results and discussion

3.1 pH, titratable acidity and salt content

Changes in pH, titratable acidity, and salt content in the fermented black pomfret during fermentation are presented in Table 2, showing that the pH decreased continuously from 6.7 to 5.3, with a significant difference between the duration of fermentation. Rhee *et al*. (2011) also reported that pH declines rapidly during 3–5 days of fermentation of *sikhae* (Korean traditional fermented marine fish) from 6.5 to less than 5.0, while the texture softened. Typically, the decrease in pH is accompanied by an increase in titratable acidity due to the production of organic acids, mainly lactic acid by LAB (Riebroy *et al*., 2007). In addition to the fermentation of the protein sources (Mahyudin *et al*., 2015), the fermentation of rice also involves changes in pH and titratable acidity (Ezzat *et al*., 2021). This rapid decrease in pH during the fermentation to produce *ikan pekasam* is beneficial since it inhibits the growth of foreign microflora (Sroka and Tuszyński, 2007; Mahyudin *et al*., 2015).

In the present study, there were significant differences in the positive and negative correlations ($p \leq 0.05$) between pH and salt content ($R = 0.996$) and titratable acidity ($R = -0.956$), with the titratable acidity increasing five-fold ($p \leq 0.05$) so the *ikan pekasam* after five weeks of fermentation had the highest titratable acidity and the lowest pH. These results indicate that the decrease in pH was associated with a decrease in salt content as well as the increased acidity of *ikan pekasam* black pomfret.

Table 2. Changes in pH, titratable acidity (% lactic acid), and salt content (%) in *ikan pekasam* black pomfret at week 0 (Control), 2, and 5 of fermentation.

Fermentation time (week)	pH	Titratable acidity (%)	Salt content (%)
0 (Control)	6.7±0.1^a	0.2±0.0^c	18.0±0.1^a
2	5.4±0.1^b	0.7±0.1^b	14.0±0.1^b
5	5.3±0.1^c	1.0±0.1^a	14.0±0.1^b

Values are presented as mean±SD (n = 3). Values with different uppercase and lowercase superscripts are statistically significantly different ($p<0.05$). ND: Not Detected.

The salt concentration in *ikan pekasam* decreased from 18.0% to 14.0% in 2 weeks, with no significant ($p > 0.05$) changes for 2 and 5 weeks of fermentation, possibly due to the leaching of salt from the fish into the fermentation liquor. The salt concentration was suitable for LAB growth as such bacteria (halophilic) are generally tolerant of high salt concentrations in the range

https://doi.org/10.26656/fr.2017.8(2).275

of 11.3% to 20.3% (Marui *et al*., 2015; Liu *et al*., 2021). Furthermore, the *ikan pekasam* produced fulfilled the Malaysian Food Regulations (1985) requirements that the salt content of *ikan pekasam* should not be less than 10%. Moreover, many gram-negative bacteria (for instance *Pseudomonas* spp.,) related to fish spoilage are halophobic and cannot replicate in salt concentrations higher than 5% (Horner, 1997), hence salt has long been used in fish preservation (Kim *et al*., 2017). Also, the salt prevents bacterial growth by lowering the water activities before fermentation (Lopetcharat *et al*., 2001; Françoise, 2010), as inadequate acid levels produced during very low-salt fermentation might lead to food poisoning (Basti *et al*., 2006; Lee and Lee, 2014).

3.2 Biogenic amine composition and content

Figure 2 (a – d) shows the HPLC chromatograms of biogenic amines in the standards, *ikan pekasam* black pomfret at 0, 2 and 5 weeks of fermentation. Biogenic amines are categorized as monoamines (histamine, tyramine, tryptamine and 2-phenylethylamine), diamines (putrescine and cadaverine) or polyamines (spermine and spermidine) (Sarkadi, 2017) based on the number of amine groups. As shown in Table 3, the total concentration of biogenic amines increased five-fold after five weeks of fermentation from 88.3 mg/kg to 434.1 mg/kg, with putrescine present in the highest concentration, followed by cadaverine. These biogenic amines were already present at significant levels at the start of fermentation. However, this outcome was lower than that observed by Rabie *et al*. (2009), who indicated that the concentration of cadaverine increased from 21 mg/kg to approximately 200 mg/kg during 20 days of fermentation of Feseekh (Egyptian salted-fermented fish). Lakshmanan *et al*. (2002) reported that putrescine and cadaverine producing bacteria survive and grow rapidly during fermentation and are associated with biogenic amine formation.

No histamine was detected at the start of fermentation, but the histamine concentration increased two-fold ($p \leq 0.05$) from 25.1 mg/kg after 2 weeks of fermentation to 50.5 mg/kg after 5 weeks of fermentation. The final histamine concentration was the hazard level for human safety set by the USFDA (50 mg/kg) (USFDA, 2021). However, in some cases, food with histamine contents of 67 and 180 mg/kg has been consumed during sensory evaluation without any symptoms of toxicity (Bulushi *et al*., 2009), as it depends on the capability of the human detoxification system (Barbieri *et al*., 2019). The tyramine concentration increased gradually 2.8-fold from 4.5 mg/kg at the start of fermentation until the fermentation process was completed, representing 5.1% of total biogenic amine content at week 5. The levels of tyramine and histamine were significantly lower compared to those found in Korean fermented and salted anchovy products (*myeolchi-jeot*) (up to 320 and 824 mg/kg, respectively) (Mah *et al*., 2002). Tyramine is mainly related to the activity of fermentative LAB, while putrescine and cadaverine are usually the results of the action of non-fermentative strains (Sarkadi, 2017).

Unlike tyramine, no 2-phenylethylamine was detected at the start of fermentation, increasing two-fold ($p \leq 0.05$) from 11.2 mg/kg after 2 weeks of fermentation

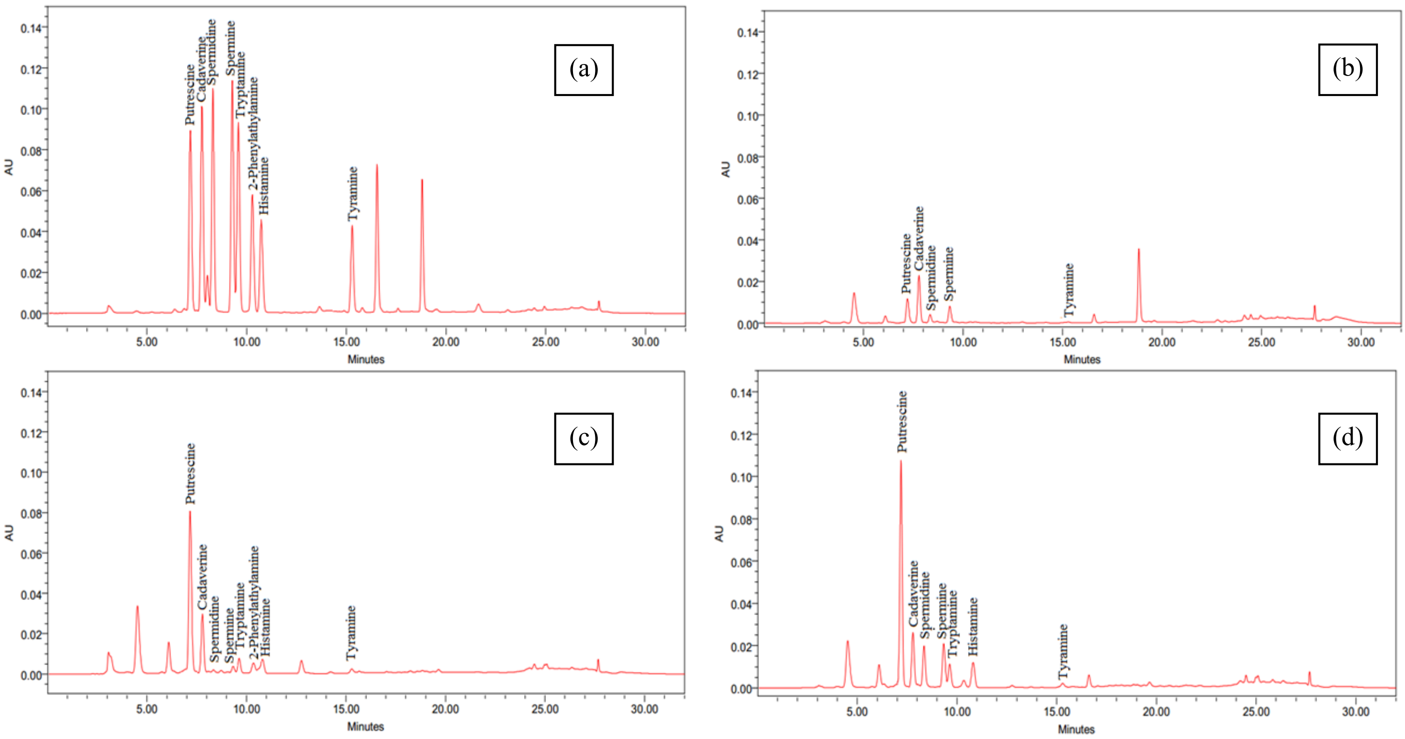

Figure 2. Typical HPLC chromatograms of biogenic amine standards (a), a sample of *ikan pekasam* black pomfret at 0 weeks (b), at 2 weeks (c), and 5 weeks (d) of fermentation.

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Table 3. Changes in biogenic amine content of *ikan pekasam* black pomfret during weeks 0 (control), 2, and 5 of fermentation.

Biogenic amine (mg/kg)	Fermentation time (week)		
	0 (Control)	2	5
Histamine	ND	25.1±2.0^{Cb}	50.5±2.3^{Ca}
Tyramine	4.5±1.3^{DEb}	11.9±0.3^{DEa}	12.5±0.9^{EFa}
Putrescine	25.7±1.7^{Bc}	158.7±1.6^{Ab}	226.1±2.4^{Aa}
Cadaverine	38.1±2.0^{Ab}	48.0±3.6^{Bb}	76.1±8.4^{Ba}
Spermidine	8.9±1.1^{CDa}	6.4±4.8^{Ea}	7.1±1.3^{Fa}
Spermine	11.1±1.5^{Ca}	13.4±0.1^{Da}	13.7±0.7^{DEFa}
Tryptamine	ND	21.7±1.3^{Ca}	26.5±2.9^{Da}
2-Phenylethylamine	ND	11.2±1.2^{DEb}	21.6±1.4^{DEa}
Total	88.3±4.5^{c}	296.3±2.5^{b}	434.1±0.3^{a}

Values are presented as mean±SD (n = 3). Values with different uppercase and lowercase superscripts are statistically significantly different (p<0.05). ND: Not Detected.

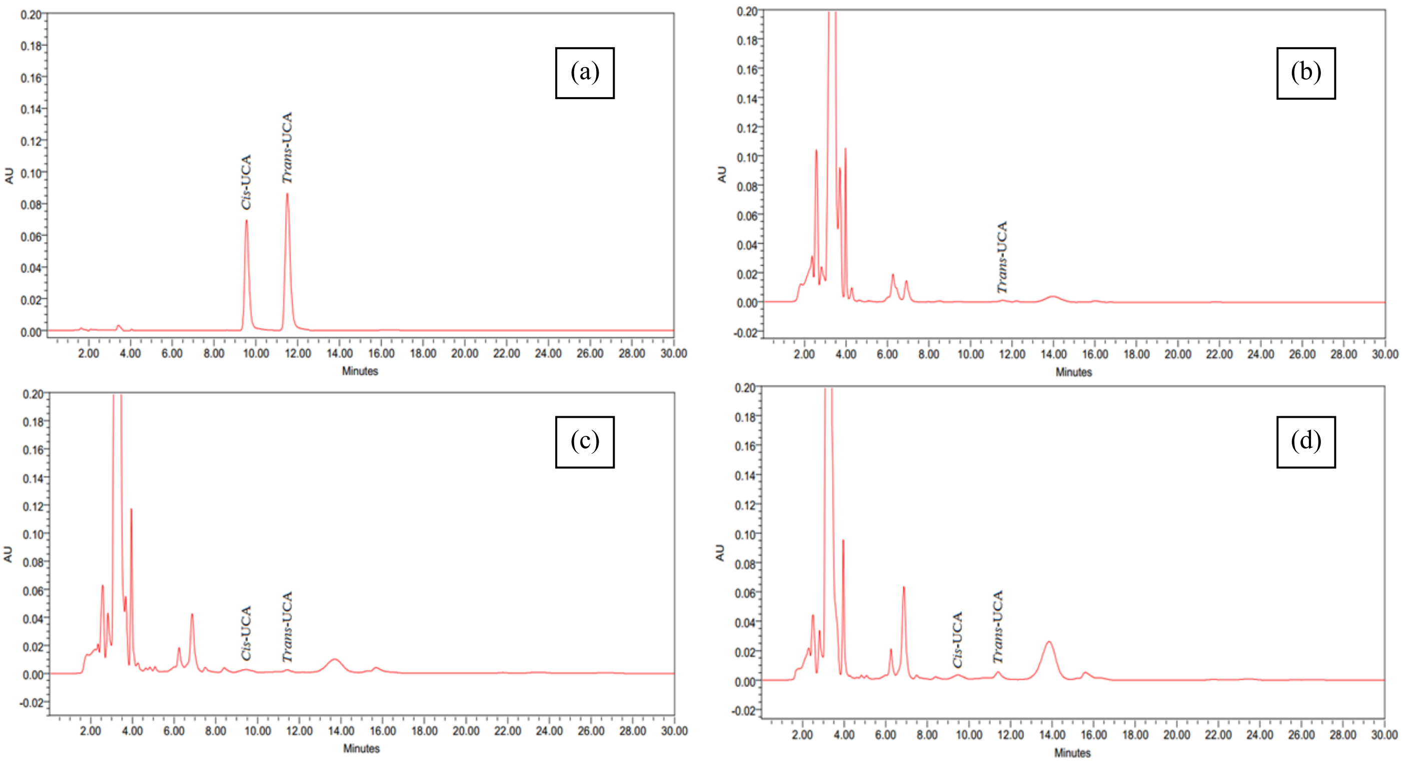

Figure 3. Typical HPLC chromatograms of *trans*- and *cis*-UCA standards (a), *trans*- and *cis*-UCA content in *ikan pekasam* black pomfret at 0 (b), 2 (c), and 5 (d) weeks of fermentation.

to 21.6 mg/kg after 5 weeks of fermentation. Likewise, no tryptamine was detected at the start of fermentation, increasing from 21.7 mg/kg after 2 weeks of fermentation to 26.5 mg/kg after 5 weeks of fermentation. Danquah *et al*. (2012) reported that spermine and spermidine are mostly found in raw meats, unlike the other biogenic amines that tend to be found in fermented products. However, the initial concentrations of these two biogenic amines did not change significantly during the fermentation but were higher than those reported by Rabie *et al*. (2009) after 20 days of ripening and storage of Feseekh. There was a strong negative correlation between pH and histamine (R = −0.904), tyramine (R = −0.979), putrescine (R = −0.968), spermine (R = −0.863), tryptamine (R = −0.992), 2-phenylethylamine (R = −0.912) and total biogenic amine (R = −0.948), suggesting that low pH promoted the microbial production of decarboxylase enzymes which act on corresponding amino acids such ornithine, lysine and histidine, thus leading to higher biogenic amine concentrations, including putrescine, cadaverine and histamine.

3.3 Composition and content of trans- and cis-UCA

UCA is an intermediate product in the metabolism of histidine. In this study, adequate separation of peaks for *trans*- and *cis*-UCA was observed, with retention times of 11.5 mins and 9.5 mins, respectively as shown in Figure 3 (a − d). *Trans*-UCA was already present in black pomfret at the start of fermentation, while *cis*-UCA was not detected, indicating that photoisomerization of *trans*-UCA into *cis*-UCA had not yet taken place. As shown in Table 4, the fermentation of black pomfret to produce *ikan pekasam* led to an increase in the total

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Table 4. Changes in *trans*- and *cis*-UCA content of *ikan pekasam* black pomfret during week 0 (control), 2, and 5 of fermentation.

Fermentation time (week)	Urocanic acid (UCA) (mg/kg)		
	Trans-UCA	*Cis-UCA*	Total UCA
0 (Control)	0.9±0.3[b]	ND	0.9±0.3[c]
2	1.1±0.1[Bb]	1.7±0.1[Ab]	2.8±0.1[b]
5	5.9±0.1[Aa]	5.0±0.1[Ba]	10.9±0.2[a]

Values are presented as mean±SD (n = 3). Values with different uppercase and lowercase superscripts are statistically significantly different (p<0.05). ND: Not Detected.

UCA (*trans*-UCA plus *cis*-UCA) after 5 weeks of fermentation. The initial concentration of *trans*-UCA increased 6.6-fold to 5.9 mg/kg during the fermentation, with a strong correlation between *trans*- and *cis*-UCA (R = 0.958, p ≤ 0.05), indicating that photoisomerization occurred to change *trans*-UCA into *cis*-UCA. Ezzat *et al*. (2015) reported a higher total UCA (12.8–28.1 mg/kg), *trans*- and *cis*-UCA (1.4–6.2 mg/kg and 6.2–22.9 mg/kg, respectively) content in *ikan pekasam* produced from black tilapia and Javanese carp that had undergone either acid-assisted or natural fermentation, which may be due to the longer fermentation period. The increase in UCA is attributed to endogenous L-histidine ammonia lyase (HAL) activity or histidase rather than the activity of microorganisms (Lehane and Olley, 2000). Furthermore, changes in the enzyme activities are probably responsible for the changes in *trans*-UCA concentrations at different fermentation times.

Ikan pekasam with a low histamine content (lower than 50 mg/kg) but higher levels of *trans*- and *cis*-UCA (occurs at low-temperature storage) is usually not rejected according to the Food and Drug Administration (FDA) of the United States of America guidance level for histamine (USFDA, 2021) since there is no proof of foodborne illness (Zare *et al*., 2013). However, Zare *et al*. (2013) also suggest that foodborne illnesses related to spoiled fish are not only generated by histamine but could be due to the existence of *trans*- and *cis*-UCA or other biogenic amines such as putrescine and cadaverine, or both.

3.4 Organic acid composition and content

Figure 4 (a – d) shows the organic acid HPLC chromatograms of standards and *ikan pekasam* samples at 0, 2 and 5 weeks of fermentation, respectively. As shown in Figure 4 (b – d), two unspecified peaks (peak a and b) were observed. Peak 'a' is a variety of peptides and amino acids such as phenylalanylglycyglycine, alanylproline and L-arginine, while Peak 'b' is leucine with substantial absorbance at 210 nm (Ezzat *et al*., 2021). The total organic acid content increased ~5.0-fold after fermentation for 5 weeks (Table 5), with lactic being the predominant organic acid, representing 38.4% and 31.5% of total organic acid content after 2 and 5 weeks of fermentation, respectively. Ezzat *et al*. (2021) also reported that lactic acid was the major organic acid in naturally fermented Javanese carp (15.0%) and black tilapia (19.7%) *ikan pekasam*. In this study, lactic acid detected at 0 weeks of fermentation may originate from the fish muscle, subsequently increasing during fermentation due to the growth of LAB (Ndaw *et al*., 2008) with a slight decline during the later stages of fermentation (5 weeks). Rhee *et al*. (2011) also reported

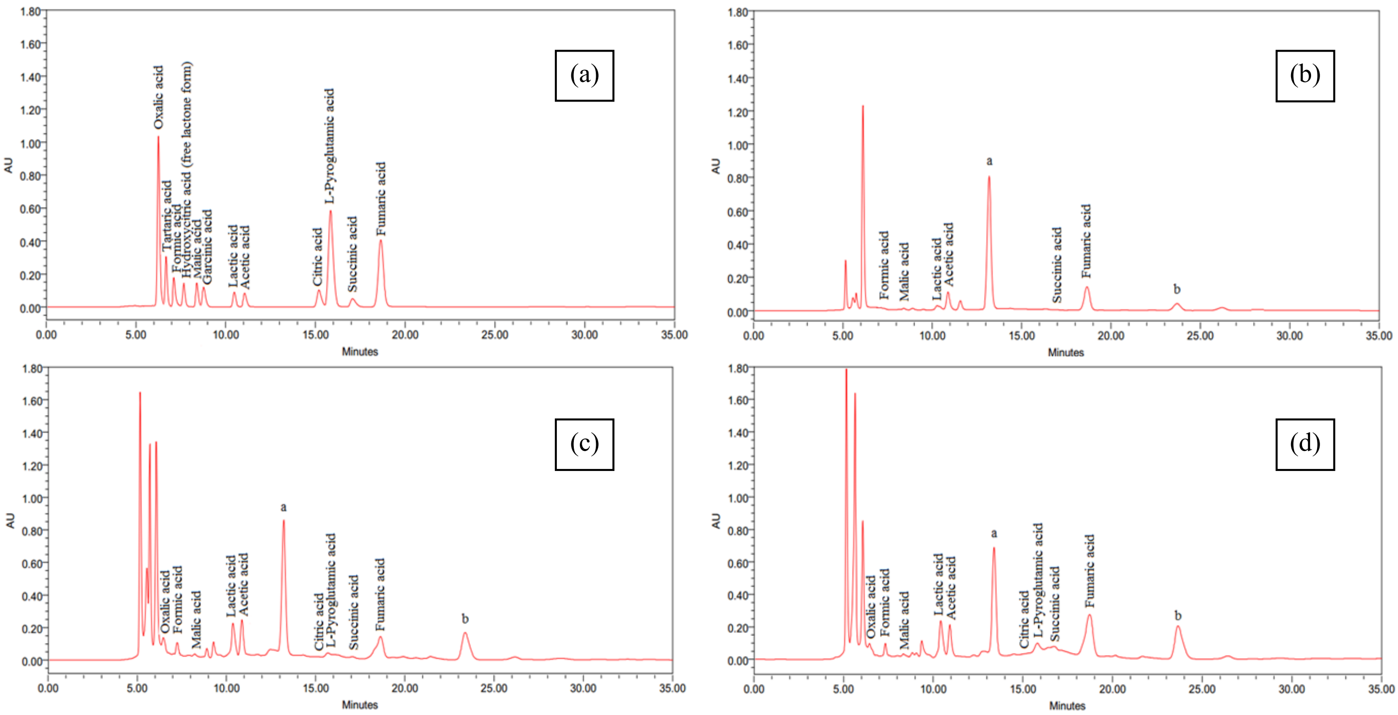

Figure 4. Typical organic acid HPLC chromatograms of standards (a) and *ikan pekasam* black pomfret samples at week 0 (b), 2 (c), and 5 (d) of fermentation.

https://doi.org/10.26656/fr.2017.8(2).275

that lipolytic bacteria declined in the initial stage of fermentation, while proteolytic bacteria such as LAB increased until Day 12 of fermentation, followed by a rapid decrease. Commonly, the flavour deterioration of fermented products is associated with the rapid growth of yeasts (Rhee *et al*., 2011).

Table 5. Changes in the organic acid content of *ikan pekasam* black pomfret at week 0 (control), 2, and 5 of fermentation.

Organic acid (mg/g)	Fermentation time (week)		
	0 (Control)	2	5
Lactic acid	2.8±0.1[Bc]	20.3±0.3[Aa]	19.0±0.3[Ab]
Tartaric acid	ND	ND	ND
Succinic acid	0.4±0.1[DEc]	3.0±0.0[Db]	11.5±0.1[Ca]
Acetic acid	6.9±0.0[Ab]	16.0±0.3[Ba]	15.4±0.2[Ba]
Formic acid	0.6±0.0[Dc]	4.7±0.1[Ca]	3.7±0.2[Eb]
Malic acid	0.6±0.2[Dc]	2.8±0.0[Db]	3.9±0.1[Ea]
Oxalic acid	ND	1.2±0.2[Ea]	0.9±0.1[Fa]
Citric acid	ND	1.5±0.1[Ea]	0.4±0.0[Fb]
L-Pyroglutamic acid	ND	0.5±0.0[Fa]	0.8±0.2[Fa]
Fumaric acid	1.9±0.0[Cb]	2.9±0.0[Db]	4.8±0.1[Da]
Total	12.2±0.1[c]	52.8±0.3[b]	60.4±0.9[a]

Values are presented as mean±SD (n = 3). Values with different uppercase and lowercase superscripts are statistically significantly different (p<0.05). ND: Not Detected.

Acetic acid was the second dominant organic acid detected (30.3% of total organic acid after 2 weeks and 25.5% of total organic acid after 5 weeks of fermentation), with the highest concentration detected after 2 weeks of fermentation. Acetic and lactic acids have been proposed as flavour enhancers (Gobbetti and Corsetti, 1997; Quitmann *et al*., 2013). The high content of lactic acid and the existence of other organic acids such as acetic and succinic acids indicate that the carbohydrates provided by ground roasted rice have been utilized by LABs in heterofermentative metabolic pathways.

Throughout the fermentation, there was no tartaric acid detected, with slight changes in the concentration of other organic acids such as succinic acid, fumaric, oxalic, malic, formic, L-pyroglutamic and citric acids. Most organic acids decreased after 2 weeks, probably due to typical acid-forming bacteria being the predominant microbes (Rhee *et al*., 2011).

The organic acids (mainly lactic acid) also decreased the pH, contributing to the flavour, nutritional, and microbial profile of fermented fish products (Liu *et al*., 2021). In this study, LAB denatures the fish muscle proteins to produce free amino acids and small-molecule peptides that contribute to the unique aroma and flavour of *ikan pekasam* black pomfret, as reported by Visessanguan *et al*. (2004) and Liu *et al*. (2021) in the production of Thai-style fermented pork sausage (Nham) and high-salt fermented Chinese fish (Suanyu), respectively.

There was a positive correlation between lactic acid and histamine (R = 0.827), tyramine (R = 0.974), putrescine (R = 0.919), spermine (R = 0.841), tryptamine (R = 0.967) and 2-phenylethylamine (R = 0.967), suggesting that LAB produced lactic acid contributing to the production of biogenic amines.

3.5 Quantitative descriptive analysis of ikan pekasam black pomfret

The five-week fermented *ikan pekasam* black pomfret was firm (Figure 5) compared to the tender/ softened muscle texture of fermented black tilapia and Javanese carp which crumbled when deep-fried (Ezzat *et al*., 2021). Also, there was a fishy, fermented rice grain odour without any pungent and ammoniacal odour as in the traditional fermented fish products in the Northern European countries including *surstromming* from Sweden, *rakfisk* from Norway, *hakarl* from Iceland (Skåra *et al*., 2015), and *hongeo* from Korea (Koo *et al*., 2016). The mean values of sensory attributes of *ikan pekasam* black pomfret after 2 and 5 weeks of fermentation are presented in Figure 6, with significant differences in most of the sensory attributes, except for tongue itchiness, which was low in both samples. Generally, the intensity of most attributes of the five-week fermented black pomfret was significantly higher (p ≤ 0.05) than the two-week sample, except for roasted

(a) (b) (c)

Figure 5. *Ikan pekasam* black pomfret after 0 (a), 2 (b) and 5 (c) weeks of fermentation.

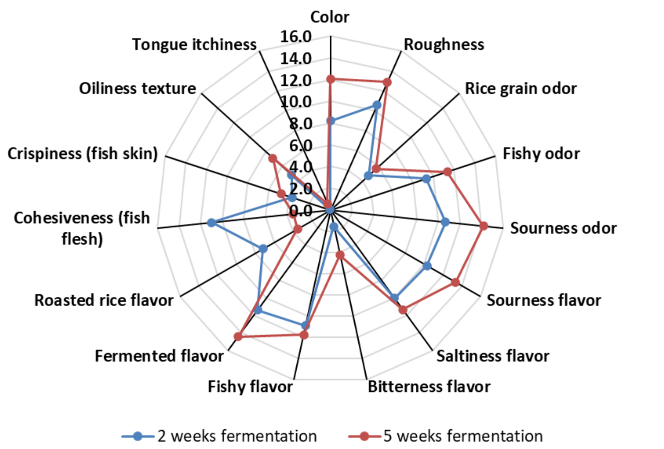

Figure 6. The intensity of sensory attributes of *ikan pekasam* black pomfret after 2 and 5 weeks of fermentation was evaluated by the trained panellists.

rice flavour and cohesiveness of fish flesh attributes. Overall, the five-week fermentation resulted in a softer texture, as well as increased flavour, odour and appearance of *ikan pekasam* black pomfret.

3.6 Hedonic scale of ikan pekasam black pomfret

The mean scores for aroma, appearance, sourness, saltiness, hardness and overall acceptability (assessed using the 9-point hedonic scale) of the fermented samples are shown in Figure 7, with the two-week fermented black pomfret having significantly higher scores in overall acceptability, sourness, and saltiness, whereas the five-week fermented black pomfret had significantly higher acceptability for overall appearance. Generally, the consumer panellists preferred the two-week fermented black pomfret. There was a significant weak correlation ($p \leq 0.05$) between the overall acceptability and saltiness ($R = 0.373$) and aroma ($R = 0.256$), suggesting that these sensory attributes have a low impact on the overall acceptability of *ikan pekasam* black pomfret. Also, there was a negative moderate relationship ($p \leq 0.05$) between overall acceptability and sourness ($R = -0.67$) and fermented flavour ($R = -0.543$), indicating that the consumers preferred the less sour and fermented flavour *ikan pekasam* black pomfret. However, there was no significant relationship ($p > 0.05$) between the lactic acid content and overall acceptability, aroma, saltiness and sourness in this study.

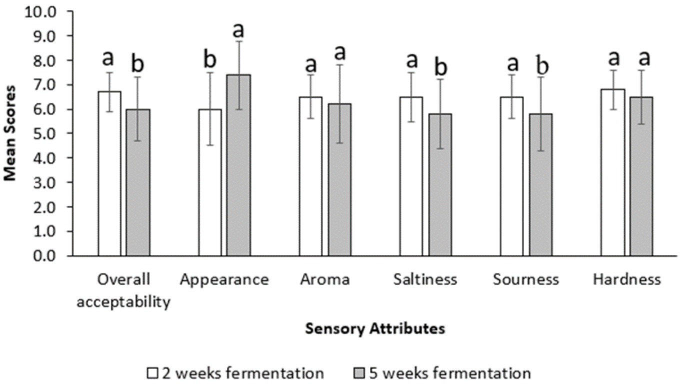

Figure 7. Sensory acceptance scores of fermented *ikan pekasam* black pomfret after 2 and 5 weeks of fermentation as evaluated by the consumer panellists. Bars with different notations are statistically significantly different ($p < 0.05$).

3.7 Principal component analysis

The score plot generated from PCA showed a relationship between fermentation time (week) and sensory evaluations (Figure 8a). The distribution of sensory evaluations is defined by the 57.5% PC1 and 10.6% PC2 (Figure 8b), where all sensory acceptability was negatively correlated to the intensity of sensory attributes. Furthermore, Figure 8c indicates that the consumer panellists preferred the two-week *ikan pekasam* black pomfret, due to its acceptable aroma, saltiness, sourness and texture.

On the other hand, Figure 9a presents the relationship between fermentation time (week), biogenic amine and UCA contents. In the loading plot graph, PC1 and PC2 accounted for 72.7% and 24.8%, respectively (Figure 9b). Overall, the biplot graph in Figure 9c revealed that two-week *ikan pekasam* black pomfret which was more preferred by the consumers had significantly lower biogenic amine and UCA contents, suggesting the production of acceptable, safer and good quality *ikan pekasam*.

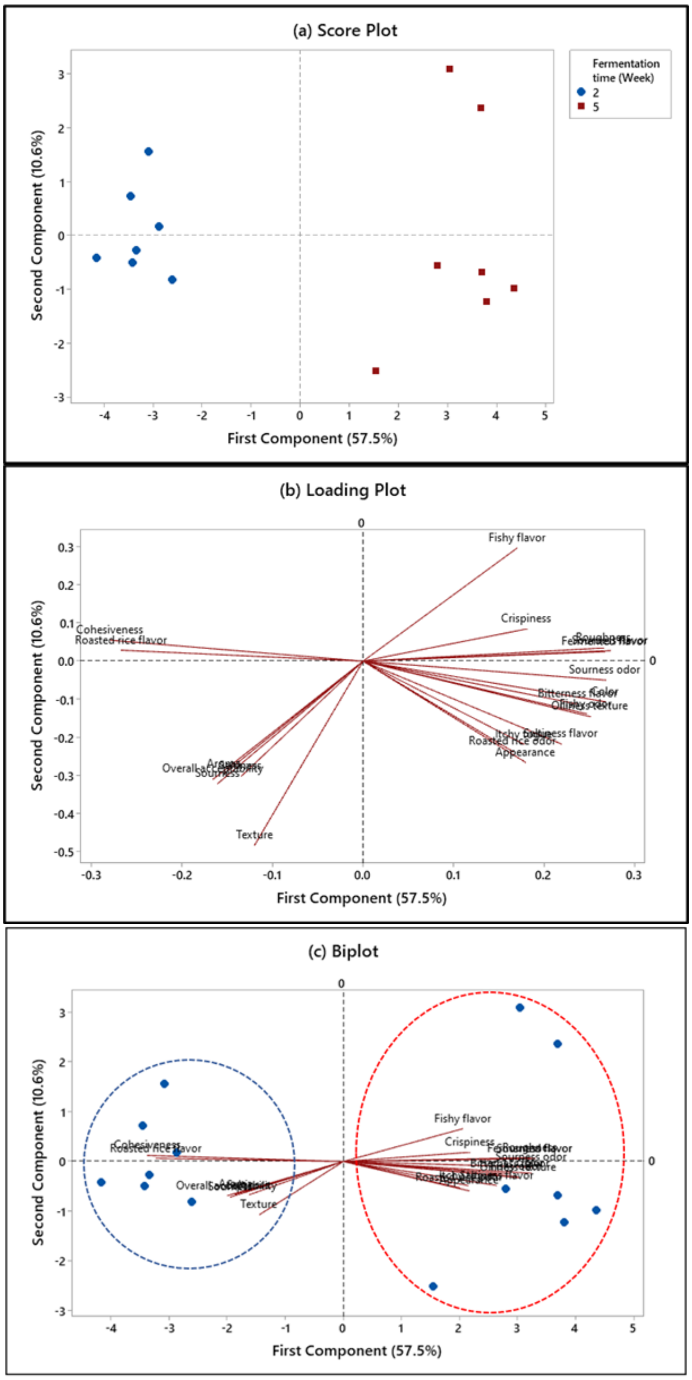

Figure 8. PCA of *ikan pekasam* black pomfret after different fermentation times. (a) Score plot of fermentation time, (b) loading plot of the intensity of sensory attributes and sensory acceptability, and (c) correlation between score and loading plots.

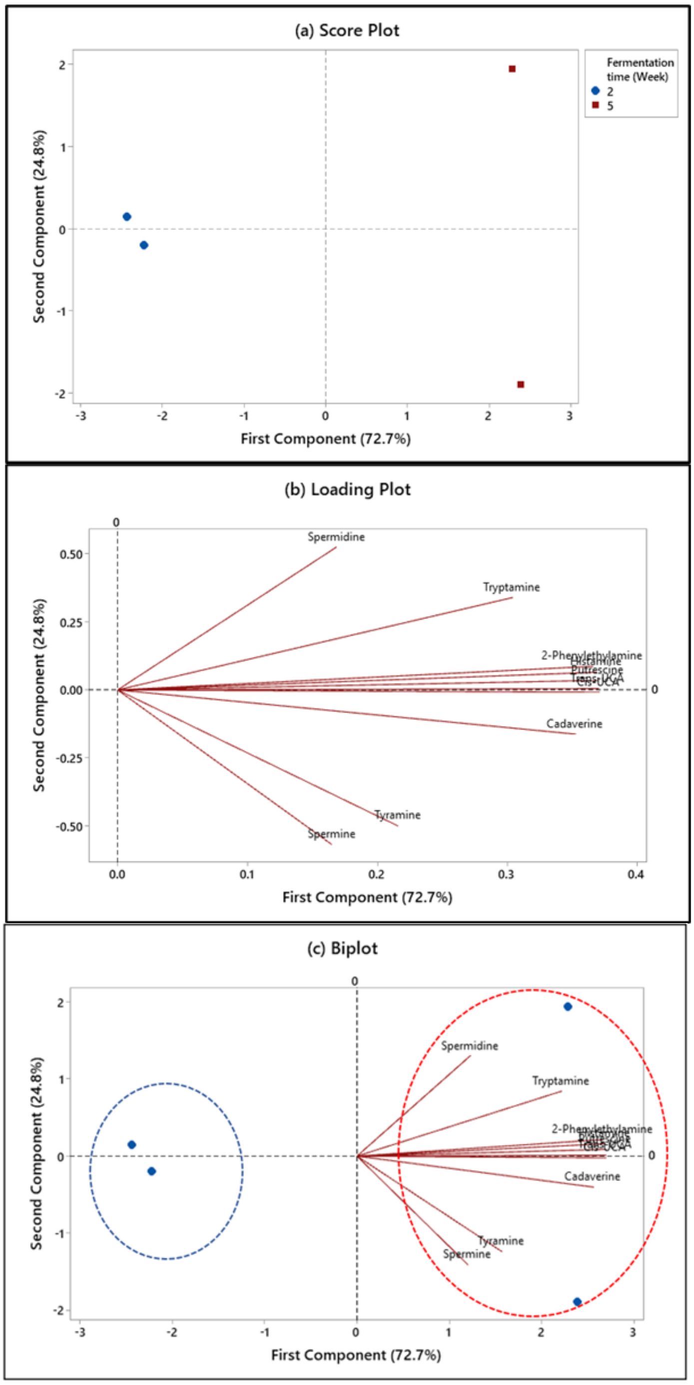

Figure 9. PCA of *ikan pekasam* black pomfret after different fermentation times. (a) Score plot of fermentation time, (b) loading plot of biogenic amine and urocanic acid contents, and (c) correlation between score and loading plots.

4. Conclusion

Titratable acidity, total biogenic amine (histamine, putrescine, cadaverine, 2-phenylethylamine), total UCA (total content of *trans*- and *cis*-UCA) and total organic acid (succinic acid, malic acid, fumaric acid) contents showed a significant increase ($p \leq 0.05$) in week 5 compared to week 2 of fermentation of black pomfret. According to the sensory evaluation accomplished by QDA, the trained panellists distinguished a significant ($p \leq 0.05$) increase of sensory attributes in 5 weeks fermented samples. However, the hedonic test found that consumers preferred the two-week fermented *ikan pekasam* black pomfret than five-week. PCA explained that this was due to the acceptable aroma, saltiness, sourness and texture of two-week *ikan pekasam*. Moreover, this preferred *ikan pekasam* had significantly lower biogenic amines and UCA contents, indicating the production of safe, good quality and acceptable *ikan pekasam*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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