Characterization of flavour and volatile compounds of fermented squid using electronic nose and HPMS in combination with GC-MS

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ABSTRACT
The present study investigated the flavour characteristics and key volatile components of fermented squid (Dosidicus gigas) as well as their formation mechanism by three different starter cultures of lactic acid bacteria (LAB) and natural squid. The sensory assessment, volatile compounds, as well as the amino acids of the mixed fermented squid were detected using pickled brine as samples of fermented squid. A total 88 types of volatile compounds were found in four samples by 0–72 h of fermentation. After fermentation by LAB, the volatile compounds of squid showed that the fermented squid with different started cultures differed mainly in terms of numbers of alcohols, ketones, and esters. Furthermore, the amounts of volatile compounds among three starter cultures were also obviously different. Gas Chromatography-Mass Spectrometer (GC-MS) analysis indicated that the mixed fermentation process contained relatively higher (+)-limonene (11.71%) (lemon scent) and 3-hydroxy-2-butanone (5.72%) (creamy taste). These compounds were not detected in the control group. The main flavour compounds of nonanal and 2, 4-decadienal were gradually reduced. Meanwhile, 2, 3-butanedione, 2-heptanone, 2-pentylfuran, and nonanoic acid are the key flavour components. The total principal component is 94.01%, which were clarified by electronic nose in combination with principal components analysis. After mixed fermentation by LAB, the total amino acid (TAA) content reached 85.36 g/100 g, which was almost twice the amount of the control, especially in aspartate, glutamate, and threonine. Our results indicated that LAB has obvious effects on deodorization and flavour promotion during the fermentation of squid. This study provided an important basis for the further development and utilization of squid.

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Introduction
Jumbo or giant squid (Dosidicus gigas) is an important cephalopod. It is one of the major invertebrate species in the Eastern Pacific, which is regarded as one of the most important ocean seafood currently. The increasing demand for giant squid meat has enhanced the economic importance of this fishery industry in the last several years due to its high content of protein, low fat, low cholesterol, and rich in resources. Squid is a high-quality protein food. It has unique sourness, forming a unique taste and flavour, which enriches the diversified development of its products. At present, squid is usually consumed as shredded squid, smoked products, or fermented squid. Fermented squid is very popular and important in the Korean and Japanese diets. During the pickling process of squid, fermentation mainly occurs through enzymatic hydrolysis by various microorganisms. However, salt has been added to extend the shelf life of squid at a very high concentration (20–30%). This may cause health problems such as...
cardiovascular and renal diseases in certain individuals. [7] Thus, consumers worldwide are becoming increasingly aware of the relationship between diet and health, and the market for so-called functional foods has been growing in recent years. [8] It was estimated that among the functional foods, probiotic food accounted for a percentage of 60–70% of the total market. [9] In this situation, low-salted and fermented squid by lactic acid bacteria (LAB) appears to be an attractive product for health-conscious consumers.

A way to prevent excessive salt intake is the application of probiotics bioprocessing. In bioprocessing, the metabolism of protein and carbohydrate is facilitated by LAB. LAB contributes to the formation of taste compounds, including amino acids and their derivatives as well as acidic components responsible for overall squid quality. [10–13] However, research on fermentation in squid by LAB has not been widely conducted yet. [14,15] LAB occurs naturally in traditional fermented dairy products and gut of many mammals. [16] Fermentation performance of *Lactobacillus paracasei* or *Lactobacillus fermentum* is similar to that of *Lactobacillus sake*, which has good acid resistivity and bile resistance. [17] Furthermore, they can secrete protease to hydrolyse protein into amino acids which, can be easily absorbed by the human body. [18] Protein hydrolysis is an essential step in the fermentation process and depends primarily on the activity of proteolytic enzymes present in squids. During proteolysis, flavour and aroma substances are formed. Proteolytic products formed during fermentation are composed mainly of soluble nitrogenous compounds such as amino acids, peptides, nucleotides, and their decomposition products. Fermented squid is nutritionally valuable as it contains high amounts of amino acids and minerals. Metabolism of free fatty acids by lipase and metabolism of amino acids are fundamental for the formation of organoleptic compounds. Thus, the combination of microbial and endogenous enzymes activity and chemical reactions (i.e. lipid autoxidation) are responsible for aroma formation in fermented squids. The interaction between starter cultures and environmental conditions drives the cell metabolisms towards results that can be either beneficial or detrimental to the product quality of the squid. In the first instance, these activities determine the formation of the organoleptic characteristics of the products, but they can also influence the safety and hygienic profile of the squids by counteracting pathogen growth and toxic compound accumulation.

Traditional fermented foods show an advantageous flavour and complex metabolites due to the various enzymes involved in spontaneous fermentation by a variety of microorganisms. Therefore, multi-strain fermentation is the fundamental way in the production of traditional condiments as well as new types of fermented foods. [19]

The present study aims to elucidate the characteristic flavour and key volatile compounds of fermented squid by *Lactobacillus paracasei* and *Lactobacillus fermentum* as well as their formation mechanism. Changes of these characters between the pure and mixed fermentation were investigated by electronic nose combined with the gas chromatography mass spectrometer equipped with head-space analyser (GCMS-HS). Furthermore, the nutritional values were evaluated according to the contents, types, and proportion of amino acid. The results will provide a theoretical basis for the further development and utilization of squid.

**Materials and methods**

**Sample preparation**

*Lactobacillus paracasei* and *Lactobacillus fermentum* were isolated from pickled wax gourd. They were inoculated on de Man Rogosa Sharpe (MRS) medium at 37°C for 48 h, and the number of colonies that appeared on the media were counted after 48 h of incubation at 37°C. The ketone bodies of squid provided by Ningbo FeiRun Marine Biological Technology Co., Ltd. were used as the raw material. Squids (average body length 40 ± 5 cm and weight 200 ± 10 g) were sliced at 3–5 cm length, 1–2 cm width, and 5 mm thickness, washed and gently dried with clean dressings to remove excess water.
Electronic nose analysis

The electronic nose (Germany AirSense) was used to tentatively estimate the aroma profile of the squid product after fermentation by LAB. It was conducted by the following procedure. Each squid sample (2.5 g) was prepared in a 10 mL glass vial and capped with a Teflon rubber cap. Those four vials were placed in the automatic sampler. The injection rate was 300 mL/min, the rate of carrier gas was 300 mL/min, and measurement time was 100 s. The cleaning time varied from 400 to 1000 s due to the different sample odour. The parameters were optimized in details, and each analysis was repeated five times.

GCMS-HS analysis

The extraction of volatile compounds was carried out by Solid Phase Microextraction (SPME). Briefly, 2.5 g (particle size less than 3 mm) of sliced and minced sample was transferred to an SPME vial (15 cm × 1 cm i.d.) at ambient temperature (12–15°C) within 3 min. The vial was then closed with a Teflon/silicone septum. The SPME fibre (100 μm polydimethylsiloxane (PDMS) extraction head) was chosen for the squid aroma analysis and it was inserted through the septum and exposed to the headspace of the SPME vial. Extraction was carried out at 60°C for 30 min while stirring in a water bath.

The volatile compounds were analysed using a GC-MS (Model 7890, Agilent Technologies, Palo Alto, CA, USA) equipped with a nonpolar column (J&W Scientific DB-5, 30 m, ID 0.25 mm, film thickness 0.25 μm), using helium as the carrier gas. The oven temperature was programed as follows: initial temperature 35°C for 2 min, 3°C/min to 40°C, and maintained at 40°C for 1 min, 5°C/min to 210°C, then kept at 210°C for 25 min. The flow rate of the carrier gas was 0.8 mL/min. The mass spectra conditions were as follows: electronic impact at 70 eV, emission current 200 μA, ion source temperature 230°C, scanning mass range 33–450 m/z, and detector voltage 350 V. Identification of the peaks was based on a comparison of their mass spectral with the spectral of the MAINLIB, NISTDEMO, REPLIB, and WILEY libraries. In some cases, it was conducted by comparison of their retention time with those of standard compounds.

Amino acid analysis

Amino acid compositions of squid samples were analysed using an amino acid analyser (L-8900, Hitachi, Japan) in accordance with the Chinese Standard GB/T 5009.124–2003(2004) with some modifications. Briefly, dried samples were weighed accurately and hydrolysed in 6 M HCl solution with drops of phenol for 24 h at 110°C after 10 min of nitrogen blowing. Then, 1 mL of hydrolysate was centrifuged at 6000 g for 5 min and 200 μL of supernatant was evaporated under nitrogen flow at 50°C. The residual was dissolved in 1.5 mL of 0.2 M HCl solution and passed through a 0.45 μm membrane filter. Twenty microlitres of the hydrolysates were injected into the amino acid analyser using an auto-sampler. Mixed amino acids standard with taurine standard were analysed before sampling. The amino acids were identified and quantified by comparing the peak profiles of the squid samples with standard amino acid profiles. Amino acid score (AAS) and essential amino acid index (EAAI) of the squid were calculated according to a previously reported method.

Sensory evaluation

For descriptive analysis of squid products, a panel of 10 trained assessors (male and female; ages 22–36 years) participated in sensory evaluation of the squid products. The participants attended weekly sensory sessions to train their ability to recognize and describe different aroma qualities. Sensory analyses were carried out in a sensory room designed for this purpose with individual
sections for each panellist. The room temperature was adjusted to 20–25°C, and analyses were carried out in tinted light.

**Statistical analysis**

Electronic nose measurements were performed using Win Muster for response analysis, load analysis, and principal component analysis (PCA). All the data were analysed using the one-way analysis of variance (ANOVA) model of Statistica 6.1 (StatSoft Italy srl, Vigonza, Italy) to evaluate significant difference among means.

**Results and discussion**

**Electronic nose analysis**

PCA is used to visualize the resemblance and difference among various measurement data for constructing the data matrix. The squid samples were separated along the first Principal component (PC), which described 69.61% of the peak variations (Figure 1, PCA) and showed four defined groups. Along the PC1 axis, the control group (unfermented squid) was located with high positive scores, and along the PC2 axis, it still had high positive scores, whereas the other three samples had low negative scores. The total contribution rate was 94.01%, indicating information via PCA analysis could reflect the difference of squid-processing modes. There was an overlap in distribution between samples Sq-LF (inoculated by *Lactobacillus fermentum*) and Sq-LF-LP (inoculated by *Lactobacillus fermentum* and *Lactobacillus paracasei*), indicating their compositions are similar. The control group was different from that of Sq-LF, Sq-LF-LP, and Sq-LP (inoculated by *Lactobacillus paracasei*). This is probably due to the fact that the growth and metabolism of LAB in the fermentation process consume the proteins, carbohydrates, fats, and other ingredients. Peptides and free amino acids produced by protein hydrolysis were the main precursors for flavour substances and volatile substances. Lipid hydrolysis resulted in the formation of oxidized products such as aldehyde, ketone, and alcohol compounds for fermented squid. Meanwhile, additives may be involved in the formation of flavour compounds in seasoning; flavour compounds with molecular interactions change the volatility, affecting food taste. In contrast to other analytical instruments, the electronic nose does not provide any identification of the compounds present. It only attempts to integrate measurements of the total headspace volatile compounds and produce an aroma pattern that will exhibit differences or similarities among samples. [22]

It is clear that the total contribution rate of the two discriminators is 94.96% (Figure 1, LDA); the contribution rates of discriminant LD1 and discriminant LD2 are 76.28% and 18.68%, respectively. It shows that the volatile flavour substances in squid processing showed an obvious distinction. The centre distance of the control group was far from the other three samples, and great changes of flavour compounds occurred among the fermented squid samples. That is due to the higher amounts of alcohol, aldehydes, ketones, volatile organic acids, and other substances produced by LAB strains. The sample of Sq-LF-LP showed a great flavour difference compared to the control group because of unique flavour substances, which eliminated a certain fishy odour. It resulted in an improved flavour quality of the product.

**Volatile compounds of squid by LAB fermentation**

Fermentation conditions can affect the differences and similarities in the volatile flavour compounds of squids. In comparison with control, fermented squid samples of Sq-LF, Sq-LP, and Sq-LF-LP have their own unique volatile compounds: 38, 35, and 38, respectively (Figure 2). Their common volatile flavour compounds were 10, 17, and 17, respectively.
The aroma profile of squids was conducted using an SPME-GC-MS technique. The relative peak area for comparison of the compound obtained from different samples is shown in Table 1. Three replicates were performed for each sample. The compounds obtained were hydrocarbons, aldehydes, ketones, alcohols, acids, esters, and others (Table 1).

Figure 1. Analyses of LDA and PCA in squid under different processing modes.

Figure 2. The variety of volatile compounds represented in a Venn diagram under different processing methods of fermented squids.

The aroma profile of squids was conducted using an SPME-GC-MS technique. The relative peak area for comparison of the compound obtained from different samples is shown in Table 1. Three replicates were performed for each sample. The compounds obtained were hydrocarbons, aldehydes, ketones, alcohols, acids, esters, and others (Table 1).
| No | Compounds                                      | Sq-LF    | Sq-LP    | Sq-LF-LP | Control  |
|----|------------------------------------------------|----------|----------|----------|----------|
| 1  | Pentane, 2-methyl-                             | -        | -        | -        | 690710   |
| 2  | Pentane, 3-methyl-                             | -        | -        | -        | 910140   |
| 3  | Cyclohexane                                    | -        | -        | -        | 2218510  |
| 4  | Methylcyclopentane                             | -        | -        | -        | 4913908  |
| 5  | Styrene                                        | -        | -        | -        | 605597   |
| 6  | Heptane                                        | 1991134a | 2411110b | 962162a  | -        |
| 7  | Benzene                                        | -        | 485809a  | -        | 769032a  |
| 8  | Myrcene                                        | -        | -        | -        | 492114a  |
| 9  | Ethylbenzene                                   | 2440770a | 818661c  | 1742568b | 5227104bc|
| 10 | (2E,4E)-2,4-Octadienal                         | 3414080a | 1203808b | 414426b  | -        |
| 11 | Tetradecane                                    | -        | 947259a  | -        | -        |
| 12 | 1-octane                                       | -        | 572302a  | -        | -        |
| 13 | Benzocyclobutene                               | 2012926a | 1243277ab| 690701b  | -        |
| 14 | Undecane                                       | 1480092a | -        | -        | 651240a  |
| 15 | Benzene,1,2,3,4-tetramethyl-                   | 85580a   | 2022869a | 148948a  | -        |
| 16 | Octadecane                                     | -        | 53528b   | -        | 323735a  |
| 17 | (+)-Limonene                                   | -        | -        | 1791943a | 492114a  |
| 18 | p-Isopropyltoluene                             | -        | 452230a  | -        | -        |
| 19 | (+)-Limonene                                   | -        | 321096b  | 93606263a| -        |
| 20 | Pentadecane                                    | -        | 428909b  | 1041439b | 3858021a |
| 21 | Hexadecane                                     | -        | -        | -        | 4420526  |
| 22 | Ethanol                                        | 2085767a | 1694725a | 56545148a| 1094088a |
| 23 | 1-Penten-3-ol                                  | 1174736a | 223269a  | 9516995a | -        |
| 24 | 3-Methyl-1-butanol                             | -        | 449685a  | 149931b  | -        |
| 25 | Hexyl alcohol                                  | 860601b  | 185894b  | 10471655a| -        |
| 26 | 1-Octen-3-ol                                   | 2236282b | -        | 4405374a | -        |
| 27 | 1-Heptanol, 4-methyl-                          | -        | 2506938a | -        | 323735a  |
| 28 | 1-Hexanol, 4-methyl-                           | -        | -        | 1325692a | -        |
| 29 | 2-Ethylhexanol                                 | 1275607b | 1320064b | 1225314  | -        |
| 30 | 1-Hexanol, 5-methyl-                           | 4087438a | -        | -        | -        |
| 31 | trans-2-Octen-1-ol                             | -        | 2871407b | 822090a  | -        |
| 32 | (Z)-Octa-1,5-dien-3-ol                         | 8350920a | 142319b  | -        | -        |
| 33 | N-octanol                                      | -        | -        | 21802612a| -        |
| 34 | Octanol                                        | 761478b  | -        | 10786771a| -        |
| 35 | Geraniol                                       | -        | 485809a  | -        | 775688   |
| 36 | Hexanalaldehyde                                | -        | -        | 1455361  | -        |
| 37 | Acetaldehyde                                   | 8491336ab| 6174406b | -        | 27143241a|
| 38 | Hexanal                                        | 9151292ab| 1612858ab| 2582282  | 3432058b |
| 39 | Heptanal                                       | -        | 711603b  | 3070701b | 84613699a|
| 40 | Octanal                                        | 5694300a | 2787534b | 4549766  | 2714368b |
| 41 | Benzaldehyde                                   | 1721554a | 86788b   | 5720014b | -        |
| 42 | (Z) – 2-heptenal                                | -        | -        | 878702   | -        |
| 43 | Nonanal                                        | 5257716ab| 2930695a | 15243554b| 1440625b |
| 44 | Decanal                                        | 1690513b | 5239741a | 16371251ab| 690710b |
| 45 | (Z) – 2-octenal                                 | -        | -        | 1507624a | -        |
| 46 | Isovaleraldehyde                               | -        | 223564c  | 9119274b | 93720453a|
| 47 | Valeraldehyde                                  | 3501126a | 1136214a | -        | -        |
| 48 | 2-Undecanone                                   | 7346716a | 1669822a | 1298953b | -        |
| 49 | 2,3-Butanediene                                | 1221209b | 832190c  | 1247011a | -        |
| 50 | 2-Butanone                                     | -        | -        | -        | 296452   |
| 51 | 3-Methyl-2-butanone                            | 1752563a | -        | 4260623a | -        |
| 52 | Acetone                                        | -        | 1631438b | 3702250b | 433362b  |
| 53 | 2-Heptanone                                    | 12753783a| 5262607a | 330842b  | -        |
| 54 | 3-Hydroxy-2-butanol                            | -        | 8419185a | 3692145a | -        |
| 55 | 2-Methyltetrahydrothioiphen-3-one              | 824491c  | 831160b  | 1263501c | -        |
| 56 | 2-Nonanone                                     | 1304096a | 5858460a | 7478452c | -        |
| 57 | 2,3-Octanediene                                | 580451b  | -        | 15394971a| -        |
| 58 | 2-Tridecanone                                  | 1425639a | -        | 2238036b | -        |
| 59 | 3-Methyl-4-pentene –2-one                     | 448399   | -        | -        | -        |
| 60 | 3-Decan-2-one                                  | 1080786  | -        | -        | -        |
| 61 | 2,3-Pentanediene                               | 1299489b | 182180b  | 232953a  | 611616b  |
| 62 | DL-2-Methylbutyric acid                        | 1174712  | -        | -        | -        |

(Continued)
Low amounts of hydrocarbons were found in the control group. High content of hydrocarbons [(+)-limonene and (-)-limonene] was detected in the sample of Sq-LF-LP. Hydrocarbons content had little difference between the samples of Sq-LF and Sq-LP, but variations could be found in the individual compounds. By contrast, aldehydes were the most representative aroma compounds in all samples. Free fatty acid contents were affected by different fermentation conditions, which consequently affected the formation of precursors of aroma compounds such as aliphatic aldehydes. During fermentation, sugar components in squid such as monosaccharides and disaccharides were decomposed to produce lactic acid via the anaerobic glycolysis pathway. A variety of enzymes, including protease, lipase, and peptidase, existed in the strain of *Lactobacillus plantarum*. The hydrolysates by enzymatic reaction of substrates such as proteins and fats in squids result in producing some flavour precursors or flavour compounds. In general, the aliphatic aldehydes derived from lipid metabolism produced various aromas such as grassy, rancid, or floral flavour depending on their concentrations. Although the relative peak area of aldehydes in total volatile compounds showed no significant differences among three fermented squid samples, variations could be found in the individual aldehydes, among which hexanaldehyde (an important aroma compound) was found only in the sample of Sq-LF-LP. Hexanal was found in higher amounts in the sample of Sq-LF-LP as compared to the control group. It indicates that a possible influence on the lipolysis and autoxidation rate occurred by the addition of starter cultures. The control group demonstrated relatively high amounts of heptaldehyde and nonanal, which were generated from polyunsaturated fatty acids by lipoxygenase and showed a fresh squid odour. Meanwhile, a fairly high concentration of isovaleraldehyde was detected on the control sample. The levels of hexanal, octanal, nonanal, and decanal detected for all samples did not show significant differences; they were all found in lower concentrations. During squid fermentation, this can be attributed to the lipase activity because of reducing the water loss and heat emission caused by microbial metabolism. It affects the gas concentration in the atmosphere closely surrounding the products. Leroy’s studies have shown that LAB regulates the composition of volatile and non-volatile compounds by degrading the free amino acids, thereby promoting the formation of squid flavour. Zhang reported the use of soluble compounds (taste) and volatile compound (aroma) to improve the flavour quality of

| No | Compounds                  | Sq-LF  | Sq-LP  | Sq-LF-LP | Control |
|----|---------------------------|--------|--------|----------|---------|
| 64 | Propiolic acid            | 4987133a | 2506938a | 905983a  | -       |
| 65 | 3-Methylbutanoic acid     | 2908708a | -       | 418952b  | -       |
| 66 | Valeric acid              | 1126852 | -       | -        | -       |
| 67 | Nonanoic acid             | -      | 1138778b | 2807063b | 1410750a|
| 68 | Hexanoic acid             | 1893718b | -       | 18067612b | -       |
| 69 | Oleic acid                | -      | 1641793 | -        | -       |
| 70 | L(+)-Lactic acid          | -      | 5267262 | -        | -       |
| 71 | Heptanoic acid            | 1299489 | -       | -        | -       |
| 72 | Octanoic acid             | -      | 611992a  | 608666b  | 1319765a|
| 73 | Acetic acid glacial       | 20857671b | 16947250b | 197558694a | 24331506b|
| 74 | Ethyl acetate             | 2818740b | 141314b  | 18063125a | 1410742b|
| 75 | Hexanoic acid hexyl ester | -      | -       | 16371251 | -       |
| 76 | 3-Methyl-1,4- Hexyl acetate| -      | 651175  | -        | -       |
| 77 | Octanoate                 | -      | -       | 4679365  | -       |
| 78 | Ethyl lactate             | -      | -       | 1495553  | -       |
| 79 | Hexyl acetate             | -      | 712561  | -        | -       |
| 80 | 1,2-Ethanediol,1-formate  | 2236282 | -       | -        | -       |
| 81 | Trimethyl amine           | -      | -       | 29655327 | 5009563 |
| 82 | Carbamic acid, monoammonium salt | - | - | - | - |
| 83 | 2-Ethylfuran              | 5973457a | 2871070a | -        | -       |
| 84 | 2-Pentylfuran             | 1174736b | -       | 4215037a | -       |
| 85 | Trans-(penten-ethyl)furan | 2818740b | 897611b  | 433342c  | -       |
| 86 | 2,6-Dimethyl pyrazine     | -      | -       | 13197611c | 1420247a|

Note: "-" represents the undetected compounds. Values with different superscripts in the same row are significantly different (p < 0.05).
fermented sausage using a strain of *Lactobacillus paracasei* as a starter by GC-MS and amino acid analyser. The results showed that the contents of glutamic acid, aspartic acid, glycine, serine, and alanine were higher than the control group without the starter. This indicates that *Lactobacillus paracasei* is a valuable strain to improve the flavour of fermented squid products. Kedia et al. (2007) reported a new type of food with unique flavour produced by yeast fermentation and LAB co-fermented in culture medium containing 5% malt suspension. The results showed that the growth of LAB was improved by the addition of yeast. In the co-fermentation process, yield of lactic acid and ethanol was increased as compared to that of LAB alone, and the pH of the fermentation broth decreased.

As compared to the control group, the levels of ketones, alcohols, and acids were significantly higher in the sample of Sq-LF-LP. One of the main reasons is the presence of high concentrations of odorant compounds such as 3-methyl-2-butanol, 2-heptanone, 3-hydroxy-2-butanol, 2-nonanone, ethanol, 1-octen-3-ol, trans-2-octen-1-ol, hexanoic acid, and acetic acid. 3-Hydroxy-2-butanol, with a pleasant buttery odour, is an important contributor to squid aroma. Moreover, some alcohols detected under the tested conditions derived mainly from lipid metabolism. 1-octen-3-ol, which imparts a strong mushroom flavour, was the most important alcohol, together with ethanol. By contrast, samples of Sq-LF and Sq-LP showed a similar change. Esters have found generally low contents in four kinds of squid samples; they are all less than 10% of the total volatile compounds.

2, 6-Dimethyl-pyrazine was detected in the sample of Sq-LF-LP and was found at a lower concentration in the control group. 2-Pentylfuran was detected in a lower concentration in samples of Sq-LF-LP and Sq-LF. They were shown to have relatively low threshold values, but identified as intense aroma-active compounds. On the other hand, they were generally appreciated for attributes such as the pleasant aroma of squids and were presumably formed by autoxidation of linoleate.

Concerning the volatile compounds, it can be generally affirmed that in the four squid samples, the main differences could be related to the starter cultures used and the fermentation conditions. The volatile compounds of squid showed that the fermented squid with different starters differed mainly in the numbers of alcohols, ketones, and esters. Furthermore, the amounts of volatile compounds among the three starters were also obviously different. The GC-MS analysis indicated that in the mixed fermentation by LAB, there were relatively higher contents of D-limonene formation with a similar lemon scent and 3-hydroxy-2-butanol formation with a similar creamy taste, whereas the main flavour compounds of nonanal and 2,4-decadienal gradually decreased. PCA analysis showed 2,3-butanedione, 2-heptanone, 2-pentylfuran, and nonanoic acid to be the key flavour components. Amino acids, including aspartate, glutamate, and threonine, were also increased. Our results indicate that LAB has obvious effects of deodorization and flavour promotion during squid fermentation.

### Amino acid analysis

Compositions of amino acids of all samples are shown in Figure 3. It has been well known that amino acids are susceptible to the processing conditions depending on the material species, amino acid type, and process method. After fermentation, the total essential amino acids contents, with the exception of leucine, valine, isoleucine, and lysine, in samples of Sq-LF, Sq-LP, and Sq-LF-LP were significantly higher than those in the control group. For non-essential amino acids, the control group had significantly higher amounts of histidine and glycine than samples of Sq-LF and Sq-LP, whereas the control group had significantly lower amounts of glutamic acid, cysteine, and aspartic acid than the fermented ones. Increase in glutamic and aspartic acids in fermented squids provided stronger umami or palatable taste, which was the characteristic flavour of monosodium glutamate. The increase of glutamic acid and aspartic acid in squid products after fermentation might be related to the flavour development of fermented squids. Samples of Sq-LF and Sq-LP possessed similar patterns in the composition and content of the amino acids.

As shown in Figure 3, the content of total amino acids (TAA) in the sample of Sq-LF-LP reached 85.36 g/100 g, which was almost twice the amount of the control group. The TAA content had a little difference between the samples of Sq-LF and Sq-LP. It is worth mentioning that a lower content of taurine...
(2-aminoethanesulphonic acid) was found in squid product by fermented processing. Taurine is a simple sulphur-containing amino acid stored in almost all animal tissues, which has beneficial roles in antihypertensive, anti-hypercholesterolemia, and anti-inflammatory functions.\[^{30}\] Some scholars found the loss of taurine in seafood products caused by soaking in brines, mincing, and washing during seafood processing.\[^{31}\] It ranges up to 100% in comparison to those in raw materials. The amount of taurine loss in food products depends on the methods of food preparation. In the present study, it can be concluded that the losses of taurine in squid products are due to some treatments such as brining, mincing, and washing before squid fermentation. Owing to the decomposition of protein caused by LAB fermentation, the flavour of amino acids in squid increased. Meanwhile, the content of essential amino acids increased and achieved digestion as well as absorption for fermented squids. These characteristics of the product indicate that fermented squid is a valuable seafood with enhanced nutrition and health care.

**Sensory evaluation**

Sensory analysis of squids fermented by the different LAB starter cultures was performed. The intensity ratings of the four samples are shown in Figure 4. The control group exhibited the lowest fishy score (3.12) among the four squid samples, whereas the yellowness index showed similar levels and reached a strong intensity rating in appearance among these samples. In comparison to the control group, there is a high score of mouthfeel for all fermented squid samples. Bitterness and astringency flavour in four samples seemed to have a lower descriptor score (<3.0) relative to the aroma and taste. Three fermented squid samples showed ordinary values of creamy taste and fat flavour, which possibly resulted from the ketones and esters produced by fermentation. The seasoning-like odour was considered a bit too strong in the food. The sample of Sq-LP exhibited a strong creamy taste, whereas highly intense fat flavour was found in the sample of Sq-LF-LP. The attributive levels of bitter flavour were generally similar among the four samples, although the sample of Sq-LF-LP showed a higher intensity of sour flavour. The sample of Sq-LF-LP exhibited lower levels of bitter flavour and astringency taste.
Conclusion

Electronic nose was successfully employed for the identification and differentiation of fermented squid products by LAB. The volatile compounds in squid samples were identified by GCMS-HS, indicating that fermented squid with different starter cultures varied mainly in the numbers of alcohols, ketones, and esters. Furthermore, the amounts of volatile compounds by the three starter cultures were also significantly different. The GC-MS analysis for the mixed fermentation process indicated that there were relatively higher contents of (+)-limonene with a lemon scent and 3-hydroxy-2-butanone with a creamy taste, whereas the main flavour compounds of nonanal and 2, 4-decadienal gradually decreased. This indicates that LAB has obvious effects of deodorization and flavour promotion during fermentation. The flavour amino acids in squid products increased via LAB fermentation, and the nutrient value of fermented squids was further enhanced. Furthermore, a number of essential amino acids improved the digestion and absorption of processed squid food. This study provided an important basis for the further development and utilization of squid resources.

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