Effect of Microbial Fermentation on the Fishy-Odor Compounds in Kelp (Laminaria japonica)

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Abstract: Kelp (Laminaria japonica) is an important marine resource with low cost and rich nutrition. However, its fishy odor has compromised consumer acceptance. In this study, the effects of fermentation with Lactobacillus plantarum FSB7, Pediococcus pentosaceus CICC 21862 and Saccharomyces cerevisiae SK1.008 on fishy notes in kelp were studied using gas chromatography-mass spectrometry (GC-MS), gas chromatography-ion mobility spectrometry (GC-IMS) and odor activity values (OAVs). Forty-four volatile organic compounds (VOCs) were identified in unfermented kelp, most of which were aldehydes, followed by alkanes, alcohols and ketones. Among them were 19 volatile compounds with OAV greater than one. Substances containing α,β-unsaturated carbonyl structure (1-Octen-3-one, (E,Z)-2,6-nonadienal, (E,E)-2,4-decadienal, etc.) are the main contributors to kelp fishy odor. The number of VOCs in kelp samples fermented by L. plantarum, P. pentosaceus and S. cerevisiae were decreased to 22, 24 and 34, respectively. GC-IMS shows that the fingerprint of the S. cerevisiae fermented sample had the most obvious changes. The disappearance of 1-octen-3-one and a 91% decrease in unsaturated aldehydes indicate that S. cerevisiae was the most effective, while L. plantarum and P. pentosaceus only reached 43–55%. The decrease in kelp fishy notes was related to the decrease in α,β-unsaturated carbonyl groups. The experimental results show that odor reduction with fermentation is feasible.

Keywords: kelp (Laminaria japonica); odor activity value; fermentation; fishy odor; gas chromatography-ion mobility spectrometry; gas chromatography-mass spectrometry

1. Introduction

Depending on different pigmentation, seaweeds are divided into red algae, green algae and brown algae [1]. Kelp (Laminaria japonica) belongs to brown algae and is rich in nutrients such as iodine, protein, alginate, glycol, amino acids and polyunsaturated fatty acids [2]. Fucoïdian, which is rich in kelp, has immune modulating, antioxidiant and anti-inflammatory effects [3]. Therefore, kelp is a “longevity food” widely consumed in East Asia [4]. The world’s leading seaweed producers are China, Indonesia and the Philippines. These three countries also have the largest number of seaweed species and the longest history of seaweed consumption [5]. In addition to being used for the production of hydrocolloids and in agriculture, most of the harvested seaweeds are used for food (75%) [6]. However, the global market share of seaweed aquaculture production for food and other uses is still less than 1% of the total biomass production [3,7].

The typical fishy odor of kelp is one of the main obstacles to its use as a bulk ingredient. Odor-causing compounds are found in many foods and can impede consumption. The beany flavor in soybeans or peas is mainly caused by aldehydes (hexanal, (E)-2-hexenal) and alcohols (hexanol, 1-octen-3-ol) [8]. (Z)-6-nonenal, (E,Z)-2,6-nonadienal, octanal, hexanal, and 6-methyl-5-hepten-2-ol contributed to the thermally sensitive aroma of fresh foods.
watermelon [9]. Ma et al. [10] found that 2-acetyl-1-pyrroline and alcohols contributed to the aroma of cooked rice; aldehydes, benzene derivatives and acids were the causes of flavor deterioration during storage. Thus far, there are few studies on the characterization of fishy odor compounds from kelp. Seo et al. [11] argue that isovaleric acid, allyl isothiocyanate, octanal and acetaldehyde are the main contributors to kelp odors. Studies by Takahashi et al. [12] have shown that 1-iodooctane, nonanal, (E)-2-nonenal, (E,Z)-2,6-nonadienal and 1-octen-3-ol are the vital main components of kombu (Laminaria spp.) odor.

Thus far, some studies have reported physical and chemical methods such as cyclodextrin embedding and flower tea cover-up to remove the fishy odor of kelp [13,14]. These means of odor removal are mainly adsorption and masking of fishy odor. However, these treatments may require special processing means and are not easily controlled [15]. The flavor of the final product could be suppressed and even introduction of other undesirable flavors could occur. As a result, the acceptability of processed kelp was decreased.

Biological deodorization is currently a reliable and efficient method. Fermentation can be used to improve the flavor of food and reduce unpleasant odors. Nedele et al. [16] investigated aroma changes by fermenting soy drink with Lycoperdon pyriforme. After 28 h fermentation, the reduction in green odorants (hexanal, (E)-2-nonenal, (E,E)-2,4-decadienal) was consistent with the sensory difference. According to the study of Yi et al. [17], fermentation by Lactobacillus plantarum could effectively remove the beany flavor components (1-octen-3-ol, hexanal and hexanol) of mung beans. After confirming that (E)-2-nonenal and (E,E)-2,4-decadienal are key aroma compounds of wheat bread crumb, Vermeulen et al. [18] used S. cerevisiae and Lactobacillus sanfranciscensis for fermentation under different conditions, and found that the transformation pathway was different. Facts have proved that microorganisms can metabolize certain ingredients in food, thereby changing the ingredients and affecting their flavor characteristics. At present, there are two main types of microorganisms used in food fermentation: yeast and lactic acid bacteria [19]. They have a wide range of fermentation applicability and good fermentation effect.

Based on the above research, the purpose of this study was to analyze the volatile organic compounds (VOCs) of kelp, find out its fishy-odor contributors, and evaluate the effects of fermentation on the fishy odorants of kelp. More importantly, the specific reasons for deodorization can be inferred by comparing the fermentation effects of different strains. This provides a basic reference for further research on the mechanism of deodorization of kelp in the future. Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-ion mobility spectrometry (GC-IMS) were used to analyze VOCs of different kelp samples. The odor activity value (OAV) is equal to the ratio of the concentration of each compound to the odor threshold, which was used to identify the substances that played an important role in kelp odor. At last, the kelp was fermented by yeast (Saccharomyces cerevisiae SK1.008) and lactic acid bacteria (Lactobacillus plantarum FSB7 and Pediococcus pentosaceus CICC 21862) to compare the deodorization effects. The results of this study may contribute to understanding the changes in fishy-odor compounds in kelp, as well as providing important implications for kelp processing.

2. Materials and Methods

2.1. Materials

Fresh kelp (Laminaria japonica) was harvested from a kelp farm (Shandong Haizhibao ocean technology Co., Ltd., Weihai, Shandong, China). The raw samples were salted and vacuum-packed, transported to the laboratory and stored at −20 °C. Salted kelp was washed three times to remove salt and impurities. After drying the surface and selecting healthy thin tissue, kelp was cut into about 1 × 1 cm square pieces. The final samples were stored in screw-capped glass flasks at 4 °C in the dark for no longer than 3 days until the next step.
2.2. Microbial Fermentation

*L. plantarum* FSB7 and *S. cerevisiae* SK1.008 were obtained from our laboratory. *P. pentosaceus* CICC 21862 was purchased from the China Center of Industrial Culture Collection (CICC, Beijing, China).

*S. cerevisiae* SK1.008 was inoculated on Yeast Extract Peptone Dextrose Medium (YPD; 10 g/L Yeast Extract, 20 g/L Peptone, 20 g/L Dextrose) agar, then the strain was grown in YPD broth at 35 °C for 18 h. Then the strain was centrifuged and diluted to 10^6 CFU/mL. The *S. cerevisiae* suspension was used as the starter, added 1% into the conical flask with the mass ratio of kelp to the water of 1:3 and fermented at 35 °C and 150 rpm for 6 h. The sample fermented by yeast *S. cerevisiae* was called YF. In contrast, unfermented kelp samples were called UF.

The *L. plantarum* FSB7 and *P. pentosaceus* CICC 21862 were cultured in MRS broth at 37 °C for 16–24 h then centrifuged and resuspended in sterilized water to obtain 10^8 CFU/mL. Inoculated into a conical flask under the same conditions as above. Inoculated samples were fermented at 37 °C in an incubator for 8 h. The samples fermented by *L. plantarum* and *P. pentosaceus* were called LF and PF, respectively.

2.3. Headspace-Solid Phase Microextraction (HS-SPME) Conditions

A manual SPME holder equipped with 1 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA) was conditioned at GC injector at 250 °C for 30 min before extraction. The final HS-SPME process contained the following steps: 5 g sample was placed in 20 mL vial attached with a top hole-cap with a PTFE/silicone septum (Shendi Glass Instrument Co., Ltd., Shanghai, China) then put on a magnetic stirrer (RCT Basic, IKA, Aachen, Germany). After being incubated at 60 °C degrees for 15 min, the SPME fiber was exposed to the kelp headspace for 30 min for extraction and then desorbed in the GC injection port at 250 °C for 5 min under splitless conditions.

2.4. GC-MS Analysis

Finnigan Trace GC ultra-chromatograph with a TSQ Quantum XLS mass detector (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a fused polar capillary column (DB-WAX, 30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA) was used.

Instrument parameters were set in reference to López-Pérez et al. [20]. The oven temperatures were programmed starting at 50 °C for 2.5 min, followed by increases to 90 °C at 3 °C/min, 140 °C at 3.5 °C/min, 180 °C at 5 °C/min, 240 °C at 15 °C/min, and finally was held at 240 °C for 10 min for column cleanliness. Helium was used as a carrier gas with a constant flow of 1 mL/min. The MS detector was operated in the full scan mode at 70 eV electron ionization, data were collected at 1.74 scans/s over the m/z range of 33 to 300 amu. A series of C7-C40 n-paraffins (Sigma-Aldrich, St. Louis, MO, USA) were injected to obtain retention index (RI).

The mass spectra of the collected aroma substances were compared with the NIST 11 and Wiley mass spectral libraries to identify volatile compounds. Additionally, the RI was compared with the reported literature, and 2,4,6-Trimethylpyridine (10 μL, 4.585 μg/mL in anhydrous alcohol) was used as an internal standard for semi-quantitative analysis.

2.5. OAV Calculation

The contribution of each volatile compound was usually evaluated by OAV; it was calculated according to

\[
\text{OAV} = \frac{C}{\text{OT}}
\]

where C is the concentration of the volatile compound and OT represents the odor threshold reported in the literature. Compounds with a threshold higher than 1 were considered to have significant effects on aroma composition. For compounds with multiple thresholds, the most recent data were selected.
2.6. Headspace-Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) Analysis

A GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a DB-WAX capillary column (60 m × 0.25 mm × 0.25 µm) and IMS instrument (FlavourSpec®, Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany) was used.

Each kelp sample (5 g) was placed into a 20 mL headspace vial and incubated at 60 °C for 15 min. Then, 1 mL of headspace gas was injected into the injector (80 °C, splitless mode) utilizing a heated syringe at 65 °C. Oven temperature started at 50 °C for 2.5 min, followed by increasing to 90 °C at 3 °C/min, 180 °C at 4 °C/min, 230 °C at 20 °C/min and finally was held at 230 °C for 15 min. The ionization source of the IMS was tritium $^3$H which provided radiation energy of 6.5 KeV. The ions were placed into a drift tube (9.8 cm length) through a shutter grid, which was operated at constant voltage (500 V/cm) and temperature (45 °C). The drift gas (nitrogen) flow was set at a constant flow rate of 150 mL/min. All analyses were performed in triplicate. N-ketones C4–C9 (Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China) were used to calculate the RI of volatile compounds. The qualitative analysis of volatile compounds was conducted based on the GC-IMS and NIST database built in GC × IMS Library Search.

2.7. Data Analysis

For GC-MS, raw data were acquired using Xcalibur software (version 1.4 SR1-Thermo Fisher Scientific, Inc.). For GC-IMS, semi-quantitative and qualitative analysis was completed by Laboratory Analytical Viewer (LAV) software and all images were generated by Gallery Plot plug-in. The cluster heat map and the principal component analysis (PCA) were produced by Origin 2018 software. One-way analysis of variance (ANOVA) with Duncan’s multiple range test was carried by SPSS Statistics 26 (IBM Corporation, Armonk, NY, USA). The significance of difference was set as $p < 0.05$. The results are presented as means ± standard deviation (SD).

3. Results and Discussion

3.1. HS-SPME-GC-MS Analysis

3.1.1. Identification of Volatile Compounds in Kelp

Volatile compounds in kelp were detected using HS-SPME-GC-MS. A total of 44 VOCs (Table 1) could be classified into eight families, which include eighteen as aldehydes, nine as alcohols, seven as ketones, one as furan, two as esters, four as halogens, two as alkanes and one as alkene. The GC profiles of the different kelp samples can be seen in the Supplementary Data (Figure S1). López-Pérez et al. [21] studied the VOCs of seven species of seaweed, most of which were coincident with ours. As shown in Table 1, aldehydes, particularly unsaturated aldehydes were the most abundant compounds, followed by alcohols and ketones.

Regarding the aldehydes family, hexanal, ($E$)-2-octenal, ($E$)-2-nonenal and nonanal were most abundant, this result was similar to Ferraces-Casais et al. [22]. Alcohols came next in content to aldehydes, 1-octen-3-ol was the most abundant alcohol in kelp, and after that were ($E$)-2-octen-1-ol and 2-non-1-ol. Takahashi, et al. [12] identified volatile compounds of dried kombu (Laminaria spp.) by GC-MS and GC-sniffing, and they believe that nonanal, ($E$)-2-heptenal, ($E$)-2-octenal, ($E$)-2-octen-1-ol and 1-octen-3-ol constitute the kombu odor. The ketones detected in the kelp were mainly trans-$\alpha$-ionone, ($E,E$)-3,5-octadien-2-one and 1-octen-3-one. Ketones usually have a distinctive odor. The ionones are 13-carbon compounds with a violet and woody aroma. Found in many fruits and flowers, they are products of the oxidative 9,10 bond-degradation of carotenoids [23]. The high levels of carotenoids in kelp produce a large amount of $\alpha$-ionone, resulting in the unique floral fragrance of kelp.
Table 1. Volatile compounds identified in different treatment kelp samples by gas chromatography-mass spectrometry (GC-MS).

| No. | Compound                        | Rt a (min) | RI b  | Concentration (µg/kg) | Cal c | Ref d | UF | YF | PF | LF |
|-----|---------------------------------|------------|-------|-----------------------|-------|-------|----|----|----|----|
|     | Aldehydes                       |            |       |                       |       |       |    |    |    |    |
| 1   | Hexanal                         | 4.46       | 1087  | 1089                  | 35.56 | 5.01  | 65.87 | 65.34 |
| 2   | (E)-2-pentenal                   | 5.46       | 1133  | 1130                  | 2.19  | ND    | 4.38  |
| 3   | Heptanal                         | 6.36       | 1182  | 1182                  | 8.11  | ND    | 12.68 |
| 4   | (E)-2-hexenal                    | 7.45       | 1212  | 1214                  | ND    | ND    | 7.44  | 7.74 |
| 5   | (E)-4-heptenal                   | 7.63       | 1239  | 1243                  | 1.87  | ND    | 2.52  |
| 6   | Octanal                          | 8.66       | 1283  | 1284                  | 7.88  | 2.19  | 12.23 | 8.43 |
| 7   | (E)-2-heptenal                   | 9.54       | 1319  | 1321                  | 20.21 | ND    | ND    | ND    |
| 8   | Nonanal                          | 11.19      | 1365  | 1385                  | 27.39 | 8.29  | 49.47 | 28.0 |
| 9   | (E)-2-octenal                    | 12.40      | 1417  | 1425                  | 96.32 | 11.65 | 58.26 | 41.14 |
| 10  | (E,E)-2,4-heptadienal            | 13.87      | 1478  | 1476                  | 6.19  | ND    | ND    | ND    |
|     | Alcohols                         |            |       |                       |       |       |    |    |    |    |
| 1   | 3-Methyl-1-butanol               | 7.22       | 1201  | 1201                  | ND    | 90.11 | ND    | ND    |
| 2   | 1-Hexanol                        | 10.22      | 1346  | 1345                  | 37.55 | 57.19 | ND    | ND    |
| 3   | (Z)-3-hexen-1-ol                 | 11.02      | 1370  | 1372                  | ND    | 0.69  | ND    | ND    |
| 4   | 1-Octen-3-ol                     | 13.18      | 1457  | 1455                  | 67.87 | 34.80 | 10.28 | 11.43 |
| 5   | 1-Heptanol                       | 13.66      | 1451  | 1453                  | ND    | 23.42 | ND    | ND    |
| 6   | 2-Ethyl-1-hexanol                | 14.02      | 1485  | 1486                  | 78.63 | ND    | ND    | ND    |
| 7   | 1-Phenyl-1-decanol               | 15.44      | 1518  | -                     | ND    | ND    | 3.52  |
| 8   | 1-Octanol                        | 15.69      | 1543  | 1540                  | 29.54 | 35.80 | ND    | ND    |
| 9   | 1-Nonen-3-ol                     | 16.01      | 1554  | 1555                  | ND    | 0.34  | ND    | ND    |
| 10  | (Z)-2-octen-1-ol                 | 17.32      | 1604  | 1605                  | 53.07 | 13.76 | 4.85  |
| 11  | 1-Nonanol                        | 18.41      | 1646  | 1653                  | 50.39 | 40.06 | ND    | ND    |
| 12  | Z-4-Dodecanol                    | 18.52      | 1630  | -                     | 1.14  | 10.69 | ND    | ND    |
| 13  | Z-2-Dodecanol                    | 19.15      | 1652  | -                     | 0.74  | ND    | 6.80  | 4.71 |
| 14  | (Z)-3-nonen-1-ol                 | 19.60      | 1687  | 1688                  | ND    | 25.22 | ND    | ND    |
| 15  | (E)-2-nonen-1-ol                 | 19.96      | 1704  | 1703                  | 84.99 | ND    | ND    | ND    |
| 16  | (E)-6-nonen-1-ol                 | 19.97      | 1710  | 1714                  | ND    | 9.30  | ND    | ND    |
| 17  | 1-Decanol                        | 21.01      | 1760  | 1763                  | ND    | 3.04  | ND    | ND    |
| 18  | (Z)-5-decen-1-ol                 | 24.31      | 1886  | -                     | ND    | 10.69 | ND    | ND    |
| 19  | Phenylethyl alcohol              | 26.25      | 1928  | 1932                  | ND    | 20.25 | ND    | ND    |
|     | Ketones                          |            |       |                       |       |       |    |    |    |    |
| 1   | 3-Octanone                       | 8.05       | 1248  | 1248                  | ND    | 14.46 | ND    | ND    |
| 2   | 1-Octen-3-one                    | 8.98       | 1296  | 1295                  | 39.57 | ND    | 32.43 | 29.48 |
| 3   | 1-Hepten-3-one                   | 9.15       | 1303  | 1303                  | 2.41  | ND    | 3.16  |
| 4   | 2,3-Octanediene                  | 9.68       | 1326  | 1325                  | ND    | 9.83  | ND    | ND    |
| 5   | 6-Methyl-5-hepten-2-one          | 9.79       | 1329  | 1330                  | 2.94  | 2.52  | 2.10  |
| 6   | 4-Octen-3-one                    | 10.57      | 1360  | -                     | 1.19  | ND    | ND    | ND    |
| 7   | (E,E)-3,5-octadien-2-one         | 16.18      | 1562  | 1562                  | 108.44 | ND    | ND    | ND    |
| 8   | (E,6,10-dimethyl-5,9-undecadien-2-one) | 23.39 | 1848 | 1849 | 19.81 | ND | ND | ND |
| 9   | trans-4-Ionone                   | 25.5       | 1926  | 1926                  | 204.63 | 35.06 | 28.38 | 17.37 |
|     | Halogens                         |            |       |                       |       |       |    |    |    |    |
| 1   | 1-Iodo-propane                   | 2.89       | 956   | 965                   | 4.27  | ND    | ND    | ND    |
| 2   | 1-Iodo-pentane                   | 5.42       | 1137  | 1164                  | 14.64 | 13.26 | 7.03  | 9.43 |
| 3   | 3-Bromo-pentane                  | 9.50       | 1317  | -                     | 13.65 | ND    | 39.0  | 30.88 |
| 4   | 1-Iodo-heptane                   | 10.33      | 1350  | 1384                  | 4.11  | ND    | 6.76  | 3.55 |
| 5   | 1,4-Dichloro-benzene             | 13.1       | 1452  | 1450                  | ND    | 17.47 | ND    | ND    |
|     | Alkanes                          |            |       |                       |       |       |    |    |    |    |
| 1   | Tetradecane                      | 14.01      | 1408  | -                     | 108   | ND    | ND    | ND    |
| 2   | Pentadecane                      | 14.12      | 1494  | 1500                  | 344.44 | 27.42 | 30.10 | 20.16 |
Table 1. Cont.

| No. | Compound                        | Rt a (min) | Cal c | Ref d | UF | YF | PF | LF |
|-----|--------------------------------|------------|-------|-------|----|----|----|----|
| 1   | 2-Pentyl-furan                  | 7.20       | 1220  | 1222  | 24.31 | 12.60 | 17.58 | 13.52 |
| 1   | 1,4-Octadiene                   | 22.88      | 1799  | -     | 7.81 | ND | ND | ND |
| 2   | 1-Tridecyne                     | 23.87      | 1873  | -     | ND | 8.33 | ND | ND |
| 3   | 5-Ethyl-1-nonene                | 24.58      | 1899  | -     | ND | 15.47 | ND | ND |
| 1   | 1-Octen-3-ol-acetate            | 10.75      | 1367  | -     | 3.10 | ND | ND | ND |
| 2   | Nonanoic acid, methyl ester     | 14.92      | 1492  | 1491  | 13.71 | ND | ND | ND |
| 3   | Decanoic acid, methyl ester     | 17.01      | 1597  | 1599  | ND | 9.68 | ND | ND |
| 1   | Oxalic acid                     | 2.66       | 937   | -     | ND | 31.31 | ND | ND |
| 1   | p-Cymene                        | 7.98       | 1271  | 1268  | ND | ND | 6.81 | 5.84 |

a The retention time of volatile compounds on DB-Wax columns. b The retention index (RI). c The retention index was calculated against n-alkanes C7-C40 on DB-Wax columns. d Reference RI (DB-WAX column) were published on NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/; accessed on 26 August 2021) and ChemSpider (http://www.chemspider.com/; accessed on 28 August 2021). “-” means there is no RI in the library. e Not detected in the sample. UF means unfermented kelp; YF means Saccharomyces cerevisiae fermented kelp; PF means Pediococcus pentosaceus SK1.008 fermented kelp; LF means Lactobacillus plantarum FSB7 fermented kelp.

3.1.2. Identification of Volatile Compounds in Fermented Kelp

As shown in Table 1, the number of VOCs in three fermentation samples decreased. Compounds in yeast S. cerevisiae SK1.008 fermented kelp sample (YF) were decreased to 33 species with the number of aldehydes greatly reduced, and alcohols became the most abundant compounds. Yeast metabolism can produce higher alcohols; they are considered to be a family of aroma compounds [24]. Some specific amino acids, such as proline and leucine, can increase the corresponding production of higher alcohols such as 3-methylbutanol, which were not detected before. In YF, most of the aldehydes disappeared, and (E)-2-nonenal and (E)-2-octenal decreased by about 90%. Polyunsaturated aldehydes are known to be degradation products of unsaturated fatty acids. Again, trans-á-ionone was still the most abundant of the ketones in YF. Certain odorous ketones, such as (E,E)-3,5-octadien-2-one and 1-octen-3-one were missing; instead, 3-octanone and 2,3-octanediene were produced.

Volatile substances in P. pentosaceus CICC 21862 fermented kelp (PF) were also greatly reduced, but the content of aldehydes was still high. Hexanal was 1.85 times compared with that in the unfermented sample, while the content of nonanal, octanal, heptanal and (E)-4-heptenal showed different degrees of increase. The signal intensity of 1-octen-3-one, trans-á-ionone and 6-methyl-5-hepten-2-one were weak. Enals and enones usually have unpleasant odors, and given that their levels do not drop much, it is clear that PF still has a strong seaweed fishy odor. When it comes to L. plantarum FSB7 fermented kelp (LF), the results were almost the same with PF.

3.2. OAV Analysis of Key Fishy-Odor Compounds in Kelp

The odor descriptions, odor thresholds and corresponding OAV were listed in Table 2. We arranged by OAV of unfermented kelp, where an OAV greater than one indicates that the substance contributes to the composition of kelp odor profile. There were 19 substances in UF with an OAV greater than one.
Table 2. The odor activity values (OAVs) and odor description of volatile compounds detected in kelp samples.

| No. | Compound | Odor Description | Odor Threshold *c* (µg/kg) | OAV | UF | YF | PF | LF |
|-----|----------|------------------|-----------------------------|-----|----|----|----|----|
| 1   | 1-Octen-3-one | metallic, mushroom, dirt | 0.01 | 3957.2 | 0 | 3242.8 | 2948 |
| 2   | (E,Z)-2,6-nonadienal | fatty, green, wax, aldehyde, deep fried, fried fat, oily | 0.02 | 2012.7 | 109.1 | 458.6 | 207.7 |
| 3   | (E,E)-2,4-decadial | cedar, floral, artificial raspberry, cooked carrots, violet | 0.07 | 1353.6 | 72.5 | 329.1 | 205.8 |
| 4   | trans-4-Ionone | deep fried fat, fatty, fried potato, oily, soapy | 1.5 | 45.2 | 23.2 | 6.9 | 7.6 |
| 5   | (E)-2-nonanal | deep fried fat, fatty, fried potato, oily, soapy | 3 | 32.1 | 3.9 | 19.4 | 13.7 |
| 6   | 2,4-Nonadienal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 0.04 | 60.2 | 0 | 78.9 |
| 7   | (E,E)-2,4-heptadienal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 0.3 | 50.5 | 0 | 0 |
| 8   | (E)-2-octanone | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 0.78 | 13.9 | 0 | 0 |
| 9   | 2-Octanol | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 0.9 | 8.8 | 2.4 | 13.6 | 9.4 |
| 10  | (E)-2-nonanone | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 5 | 7.1 | 1 | 13.2 | 13.1 |
| 11  | 1-Tridecanone | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 6 | 4.1 | 2 | 2.9 | 2.3 |
| 12  | 1-Octen-3-one | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 3 | 2.7 | 0 | 2.8 | 4.2 |
| 13  | (Z)-2-octanal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 45.5 | 1.1 | 0.9 | 0 |
| 14  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 75 | 0.7 | 0.2 | 0.1 |
| 15  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 130 | 0.7 | 0 | 0 |
| 16  | (E)-2-octen-1-ol | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 15 | 1.6 | 0 | 0 |
| 17  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 60 | 0.3 | 0 | 0 |
| 18  | (E)-3,5-octadien-2-one | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 15 | 0.2 | 0 | 0 |
| 19  | (E,E)-2,4-heptadienal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 250 | 0.2 | 0 | 0 |
| 20  | (E)-2-heptanal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 1280 | 0.1 | 0 | 0 |
| 21  | (Z)-2-heptanal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 50 | 0.1 | 1 | 0 |
| 22  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 75 | 0.7 | 0.2 | 0.1 |
| 23  | (E)-2-octanal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 350 | 0 | 0 | 0 |
| 24  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 17 | 0 | 0 | 0.4 | 0.5 |
| 25  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 41 | 0 | 0 | 0.2 | 0.1 |
| 26  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 250 | 0.4 | 0 | 0 |
| 27  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 200 | 0 | 0 | 0 |
| 28  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 330 | 0.1 | 0 | 0 |
| 29  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 1 | 9.3 | 0 | 0 |
| 30  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 47 | 0 | 0.1 | 0 |
| 31  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 60 | 0.3 | 0 | 0 |
| 32  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 23 | 0 | 0.6 | 0 |
| 33  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 90 | 0 | 0 | 0 |
| 34  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 12 | 0.8 | 0 | 0 |
| 35  | (Z)-3-hexenal | fatty, fruity, grass, mushroom, raw mushrooms, sweet | 0.01 | 0 | 0 | 681.5 | 584.2 |

* Rank in descending order according to the OAV values of UF.  
  a Odor description refers to Fenaroli's Handbook of Flavor Ingredients [25] and The LRI and Odour Database (http://www.odour.org.uk/; accessed on 26 August 2021).  
  b Odor threshold in water. The values were according to the reported and Compilations of odor threshold values in air, water and other media [26].
At the top of the list is 1-octen-3-one, because it has a high content, a low threshold (0.01 µg/kg) and also the smell of metallic, mushroom, dirt. Therefore, it can be postulated that 1-octen-3-one is the main component of the fishy odor of kelp. This ketone is also known to be the degradation product of unsaturated fatty acids by chemical autoxidation reactions [27,28]. A unique phenomenon in the YF sample is that the disappearance of 1-octen-3-one is accompanied by the generation of 3-octanone, which is consistent with the results of Wanner and Tressl [28] and La Guerche et al. [29]. In their study, reductases extract of S. cerevisiae irreversibly catalyzing the enantioselective reduction of α,β-unsaturated carbonyl and the conversion ratio of 1-octen-3-one catalyzed to 3-octanone reached 90%. It was later confirmed that it was enone reductases or enoate reductases [EC 1.3.1.31]. They predominantly belonged to the “Old Yellow Enzyme” family of flavin and NADPH-dependant reductases [30]. This is why many aldehydes and ketones containing α,β-unsaturated carbonyl in YF samples have been reduced and their contents were significantly decreased. 1-Octen-3-ol is another contributor; it has a fatty, grass and mushroom flavor. Studies have shown that 1-octen-3-ol can be derived from the oxidative decomposition of linoleic acid with 10-hydroperoxide [31].

Unsaturated aldehydes (E,Z)-2,6-nonadienal, (E,E)-2,4-decadienal and (E)-2-nonenal ranked second, third and fifth. The 2-alkenals and 2,4-alkadienals have extremely low odor thresholds, so they play an important role in the overall odor profile. Aldehydes usually have a green, fatty, tallow odor; in sensory science, these flavors are called aldehyde flavors. With the increase in C-chain length, the odor threshold decreases and the odor becomes less citrusy, more fat-like [32]. These aldehydes are mainly produced by lipid oxidation, which may be related to a large number of lipids in kelp. According to literature reports, lipoxigenase in soybeans and peas catalyze unsaturated fatty acids into aldehydes [33]. In cereals, unsaturated fatty acids are oxidized by lipoxigenase during crushing or grinding, and then decomposed into aldehydes [34]. The decrease in the content of unsaturated aldehydes was also due to the effect of enone reductases.

Substances containing α,β-unsaturated carbonyl structure, such as 1-octen-3-one, 2,4-nonadienal, cis-4,5-epoxy-(E)-2-decenal, 1-hepten-3-one, (E)-2-decenal and 2-undecenal were significantly reduced in YF, which indicated that the fishy-odor compounds were sharply reduced. The odor profiles of LF and PF were similar. The content of most of the substance content was basically unchanged. In general, the content of fishy odorants decreased slightly. This may be related to the content and activity of enone reductase in yeast and lactic acid bacteria.

3.3. HS-GC-IMS Analysis

Figure 1 is a 3D topographical visualization of volatile compounds of kelp in four different treatments (UF, YF, PF, LF). The ordinate represents the retention time of the gas chromatograph, the abscissa indicates the ion drift time after normalization and the Z-axis shows the intensity of the peak. The color depth of the point means the concentration of the substance, from blue to red, representing the concentration is getting higher [35]. In Figure 1, YF’s aroma compounds were significantly different from other samples, and the changing substances are circled.

The qualitative and quantitative results of the volatile compounds in different treatment kelp samples were shown in Table 3. Eighty-two volatile compounds from four different treatments kelp samples were listed. Most of them were identified by the GC-IMS NIST database, but there were still 13 compounds with no qualitative results due to the limited data from the library database. It was worth mentioning that some volatile compounds were repetitive, which was their monomer and dimer with similar retention time and different drift times [36].

Figure 2 is divided into three areas. Region A represents the VOCs shared by the four samples with similar contents. Area B represents the substances with more contents in YF, and contains the following substances: dimethyl disulfide, 1-hexanol-D, 3-methyl-1-butanol, limonene, 2,3-octanediene and others. Among these substances, alcohols are the
most common compounds. Alcohols are produced by the oxidation and decomposition pathways of grease and lipid, and 3-Methyl-1-butanol is the side product from the alcohol fermentation of starch and sugar. Ge et al. [37] also found that the content of 3-methyl-1-butanol was significantly increased in hot drying peppers. The thermal oxidation of polyunsaturated fatty acids or the thermal degradation of amino acids produced ketones. Fan et al. [34] and Chen et al. [38] reported an increase in 2-pentanone and 2,3-octanedione in their samples after cooking or fermentation. Additionally, 3-Methylbutanal belongs to Strecker aldehydes. The reaction to form Strecker aldehydes is one of a series of complex reactions, collectively referred to as the Maillard reaction [39]. In region C, there are the following substances: hexanal, nonanal, (E)-2-octenal, (E,E)-2,4-decadienal, octanal, phenylacetaldehyde, etc. Most of them were odor-causing compounds, with a harsh, green, unpleasant odor. The content of volatile substances in YF is significantly less than that of the other three. This result is consistent with GC-MS OAV analysis: enone reductases or enoate reductases catalyze the reduction of α,β-unsaturated carbonyl structure.

Figure 1. Gas chromatography-ion mobility spectrometry (GC-IMS) 3D topographic plot of four different treatments of kelp samples. UF: unfermented kelp; YF: yeast S. cerevisiae fermented kelp; PF: P. pentosaceus SK1.008 fermented kelp; LF: L. plantarum FSB7 fermented kelp.

In addition, there were substances not detected by GC-MS: 2,6-Dimethylpyrazine is one of the alkyl pyrazines, and it has a musty, tobacco, earthy mushroom odor. However, its threshold is high, its OAV value is low and it has little effect on the overall odor. Pyrazines and their derivatives play an important role in food aroma. Several reports have highlighted the microbial origin of pyrazine in fermented soybeans and cheese [40]. Alpha-Pinene and limonene both belong to the terpenes. Terpenes are a group of natural hydrocarbons that are widely found in plants [41]. Linalool is a kind of terpene alcohol. It is synthesized from the α-pinene or β-pinene contained in turpentine.

Figure 2. Gallery plots (fingerprints) of volatile compounds in four different kelp samples. M: monomer; D: dimer. (A–C) regions represent different volatile organic compounds (VOCs) content characteristics of UF, YF, PF and LF.
| No. | Compound                              | DT a (ms) | RT b (s) | RI c       | Comment     | Signal Intensity |
|-----|---------------------------------------|-----------|----------|------------|-------------|-----------------|
| 1   | Propanoic acid ethyl ester           | 1.1391    | 386.4    | 968.7      |             | 17,479.39 ± 1024 |
| 2   | Isovalerone                           | 1.8044    | 704.8    | 1169.4     |             | 9009.6 ± 227.5a |
| 3   | Hexanal                               | 1.5578    | 563.6    | 1096.8     | monomer     | 8524.21 ± 230.13a |
| 4   | Nonanal                               | 1.2572    | 573.4    | 1010.1     |             | 5197.54 ± 285.31b |
| 5   | alpha-Pinene                          | 1.2863    | 434.2    | 1000       | monomer     | 3617.51 ± 180.87a |
| 6   | Ethanol                               | 1.1521    | 1193.8   | 1447.7     | dimer       | 3520.98 ± 141.86a |
| 7   | Methyl isobutanoate                   | 1.1438    | 306.2    | 933.4      |             | 3057.95 ± 107.75a |
| 8   | Linalool                              | 2.2208    | 1363.2   | 1530.4     |             | 2279.88 ± 103.93a |
| 9   | 1-Butanol                             | 1.3595    | 561.6    | 1106.9     | monomer     | 1812.33 ± 61.59a |
| 10  | 2,3-Pentadione                        | 1.3063    | 481.8    | 1062.3     |             | 1283.54 ± 26.19a |
| 11  | 2-Pentanone                           | 1.1246    | 318.6    | 932.6      | monomer     | 1259.46 ± 32.67a |
| 12  | Acetoin                               | 1.1279    | 459      | 924.3      | dimer       | 1042.84 ± 40.15a |
| 13  | 2-Butanone                            | 1.2489    | 824.6    | 1230.7     | monomer     | 497.18 ± 7.47c |
| 14  | 2-Pentylfuran                         | 1.2692    | 954      | 1314.1     |             | 964.55 ± 5.55b |
| 15  | Ethanol                               | 1.1128    | 318.6    | 943.6      |             | 1012.42 ± 60.74ab |
| 16  | 2,3-Butanediene                       | 1.1772    | 472.8    | 991        |             | 1071.81 ± 31.3b |
| 17  | Area 80                               | 2.043     | 1040.2   |             |             | 1023.83 ± 25.68a |
| 18  | Valeraldehyde                         | 1.1838    | 438.4    | 997.5      | monomer     | 967.24 ± 14.53b |
| 19  | (E)-2-Octenal                         | 1.3299    | 1156.6   | 1443.8     | dimer       | 918.01 ± 23.18b |
| 20  | (E)-2-Octenal                         | 1.8139    | 1153     | 1445.7     |             | 872.69 ± 8.73a |
| 21  | Heptanal                              | 1.3259    | 741.2    | 1197.3     | monomer     | 881.88 ± 25.04a |
| 22  | Ethyl isovalerate                     | 1.6328    | 735.2    | 1198.6     |             | 757.72 ± 19.59c |
| 23  | Area 79                               | 1.3336    | 562      | 1088.1     |             | 829.7 ± 20.81a |
| 24  | Butanone                              | 1.2869    | 629.8    | 1129.1     |             | 822.54 ± 24.96a |
| 25  | (E)-2-hexenal                         | 1.5131    | 808.4    | 1233.8     |             | 805.2 ± 24.16a |
| 26  | (E)-2-heptenal                        | 1.6662    | 993      | 1338.8     | monomer     | 781.79 ± 35.64a |
| 27  | (E,E)-2,4-decadienal                  | 1.3576    | 1154.4   | 1815.7     |             | 677.47 ± 23.95a |
| 28  | 3-Methyl-3-buten-1-ol                 | 1.1785    | 812      | 1260.2     |             | 678.43 ± 48.78a |
| 29  | Area 62                               | 1.0758    | 489.2    |             |             | 693.59 ± 26.87b |
| 30  | Octanal                               | 1.4094    | 985.8    | 1294.1     |             | 610.11 ± 24.82a |

**Table 3.** Volatile compounds identified in different kelp samples by Gas chromatography-ion mobility spectrometry (GC-IMS).
| No. | Compound                      | DT * (ms) | RT b (s) | RI c | Comment      | Signal Intensity |
|-----|-------------------------------|-----------|----------|------|--------------|-----------------|
|     |                               |           |          |      |              | UF              |
| 31  | Cyclohexanone                 | 1.4496    | 946.4    | 1312.5 | monomer dimer | 516.94 ± 28.5a |
| 32  | Cyclohexanone                 | 1.5117    | 947.2    | 1313.2 |               | 500.01 ± 30.15a |
| 33  | Area 47                       | 1.139     | 484.6    |       |              | 500.08 ± 9.82a  |
| 34  | 2-Methyl-5-hepten-2-one       | 1.0887    | 1002.2   | 1340.2 |               | 474.44 ± 23.96a |
| 35  | 2-Propanol                    | 1.0859    | 293.4    | 930.8  | monomer dimer | 481.39 ± 18.94a |
| 36  | 2-Hexanone                    | 1.1888    | 490.4    | 1054.2 |               | 447.93 ± 17.98a |
| 37  | 1-Octen-3-one                 | 1.6756    | 948.2    | 1315.7 |               | 369.09 ± 8.76b  |
| 38  | Hexanoic acid                 | 1.3107    | 1768.2   | 1863.4 |               | 368.35 ± 16.55a |
| 39  | 1-Pentanol                    | 1.2483    | 819.6    | 1252.4 |               | 360.63 ± 22.33a |
| 40  | 1-Propanol                    | 1.2611    | 437.8    | 1011.7 |               | 457.04 ± 10.7b  |
| 41  | 2,6-Dimethylpyrazine          | 1.1352    | 1020.6   | 1351.7 |               | 293.16 ± 10.26a |
| 42  | 3-Methyl-1-butanol            | 1.243     | 773      | 1204.1 |               | 265.2 ± 6.7b    |
| 43  | Area 62                       | 1.1294    | 516.4    |        |              | 264.03 ± 4.05b  |
| 44  | (E,E)-2,4-heptadienal         | 1.1434    | 1193.7   | 1482.4 |               | 259.04 ± 11.52a |
| 45  | Hexanoic acid                 | 1.3107    | 1768.2   | 1863.4 |               | 263.23 ± 5.26b  |
| 46  | Area 50                       | 1.117     | 351.6    |        |              | 237.68 ± 4.75a  |
| 47  | Area 43                       | 1.1886    | 1261     |        |              | 209.37 ± 8.78b  |
| 48  | Z-4-Dodecenol                 | 1.4889    | 1258.2   | 1996.2 |               | 221.7 ± 10.69a  |
| 49  | 2,4-Nonadienal                | 1.6144    | 1197.4   | 1668.5 |               | 219.26 ± 8.66a  |
| 50  | Octylaldehyde                 | 1.3974    | 932      | 1299.3 |               | 188.96 ± 4.87a  |
| 51  | Heptan-2-one                  | 1.6268    | 733.8    | 1189.6 | monomer dimer | 190.74 ± 1.14a  |
| 52  | Heptan-2-one                  | 1.2591    | 731.2    | 1191.4 | monomer dimer | 186 ± 8.94d     |
| 53  | 1-Hexanol                     | 1.6343    | 1030     | 1405.1 | monomer dimer | 479.07 ± 5.5a   |
| 54  | 1-Hexanoyl                   | 1.3215    | 1028.8   | 1405.7 |               | 293.7 ± 14.88b  |
| 55  | Area 26                       | 1.5704    | 951.4    |        |              | 179.22 ± 5.38a  |
| 56  | Ethyl lactate                 | 1.5308    | 1022.4   | 1358.3 |               | 178.42 ± 7.23a  |
| 57  | Area 35                       | 1.1726    | 1011.8   |        |              | 169.76 ± 4.58b  |
| 58  | Benzaldehyde                  | 1.1473    | 1316.4   | 1547   |               | 176.05 ± 2.99b  |
| 59  | 3-Methylbutanoic acid         | 1.2172    | 1549.8   | 1688.5 |               | 167.62 ± 4.32b  |
| 60  | Norvaldehyde                  | 1.4688    | 1099.8   | 1386.7 |               | 166.39 ± 8.32a  |
| 61  | 2-Undecenal                   | 1.1014    | 1451.6   | 1755.8 |               | 157.99 ± 3.45d  |
| 62  | (E,Z)-2,6-nonadienal          | 1.3679    | 1405     | 1590.3 |               | 151.41 ± 3.72b  |
| 63  | cis-3-Hexen-1-ol              | 1.2591    | 1143     | 1433.4 |               | 148.18 ± 9.51a  |
| 64  | Area 66                       | 1.0893    | 380.4    |        |              | 140.43 ± 9.2a   |
| 65  | Area 76                       | 1.2492    | 833.4    |        |              | 127.03 ± 1.49b  |
| 66  | Ethyl isobutyrate             | 1.4325    | 379.8    | 983.5  |               | 121.49 ± 2.08b  |
| 67  | Phenylacetaldehyde            | 1.2622    | 1345.4   | 1648.5 |               | 104.34 ± 4.76a  |

**Table 3. Cont.**
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| No. | Compound                      | DT * (ms) | RT b (s) | RI c | Comment | Signal Intensity |
|-----|-------------------------------|-----------|----------|------|---------|-----------------|
|     |                               |           |          |      |         | UF              |
| 68  | 2-Methyl-1-propanol           | 1.1375    | 502      | 1101 |         | 93.02 ± 4.56a   |
|     |                               |           |          |      |         | YF              |
| 69  | Area 60                       | 1.1018    | 347.6    |      |         | 94.71 ± 6.81a   |
|     |                               |           |          |      |         | PF              |
| 70  | Propanoic acid                | 1.1028    | 1232     | 974.5|         | 91.15 ± 3.32b   |
|     |                               |           |          |      |         | LF              |
| 71  | 3-Methylbutanal               | 1.4013    | 379      | 920.8|         | 87.88 ± 1.32b   |
|     |                               |           |          |      |         | UF              |
| 72  | Furfural                      | 1.3234    | 1245.6   | 1472.6|         | 65.17 ± 3.26a   |
|     |                               |           |          |      |         | YF              |
| 73  | 1-Octanol                     | 1.4643    | 1319     | 1566.6|         | 61.05 ± 0.93a   |
|     |                               |           |          |      |         | PF              |
| 74  | Limonene                      | 1.2283    | 770.8    | 1212.1|         | 45.15 ± 0.76b   |
|     |                               |           |          |      |         | LF              |
| 75  | 3-Octanone                    | 1.3285    | 925.4    | 1272.8|         | 43.05 ± 1.62a   |
|     |                               |           |          |      |         | UF              |
| 76  | (Z)-3-nonen-1-ol              | 1.1696    | 1399.6   | 1685.7|         | 42.92 ± 0.43bc  |
|     |                               |           |          |      |         | YF              |
| 77  | Ethyl propanoate              | 1.1427    | 348      | 911.3|         | 41.12 ± 0.98b   |
|     |                               |           |          |      |         | PF              |
| 78  | 1-Pentanal                    | 1.2098    | 318      | 925.5|         | 36.2 ± 1.47b    |
|     |                               |           |          |      |         | LF              |
| 79  | 1,4-Dichloro-benzene          | 1.4674    | 1199.8   | 1450 |         | 31.81 ± 0.83c   |
|     |                               |           |          |      |         | UF              |
| 80  | 2,3-Octanedione               | 1.1697    | 1202.7   | 1325.6|         | 24.49 ± 0.87bc  |
|     |                               |           |          |      |         | YF              |
| 81  | Phenylethyl alcohol           | 1.4656    | 1801.5   | 1915.2|         | 20.54 ± 0.42b   |
|     |                               |           |          |      |         | PF              |
| 82  | Dimethyl disulfide            | 1.1977    | 584      | 1111.1|         | 12.38 ± 0.3b    |

a Retention indexes; b retention times; c drift times. Different letters (a, b, c, d) show significant differences at a 95% confidence level.
3.4. Analysis Based on PCA Results and Heat Map Clustering

A total of 12 sets of signal intensity data from four samples from GC-IMS were processed, dimensionally reduced and the PCA was carried out (Figure 3). These irrelevant variables obtained through the PCA can reflect the main information of the original variables. The first two principal components (67.6 and 22.9% of PC1 and PC2, respectively) explained 90.5% of the total variance, indicating that the two principal components could reveal most information of different samples. In Figure 3, four samples are well separated, forming 3 regions, which indicates that it is very good to distinguish the differences between the samples. PF and LF had similar positive and negative score values, so they had similar odor profiles. This indicated that the lactic acid bacteria had similar effects on kelp fermentation.

Figure 3. Principal component analysis (PCA) scores plot based on the signal intensity obtained from four different kelp samples.

To further study the odor profile between the kelp of different fermentation groups, heat map clustering analysis is used, which can clearly and intuitively reflect the differences. The signal strength of each volatile flavor is marked with a different color on the heat map (Figure 4). From bottom to top is blue to white and last to red, which indicates increasing peak signal intensity. PF and LF samples clustered together, which means that they had the highest correlation. Based on the vertical mode, the volatile compounds in kelps were classified into four groups according to the peak intensity. In the bottom two areas, the content of 2-propanol, 2-pentanone, 2,6-dimethylpyrazine, etc., were distributed differently in four samples due to their odor difference. The middle part of the heat map contains substances such as 1-octen-3-one, 1-octen-3-ol and many aldehydes which have unpleasant odors such as green, fatty, pungent and tallow. The compounds at the top have a mild, rose-like, buttery and fruity odor with a low odor threshold. Therefore, the YF sample had fewer fishy odors and more fruit and flower aromas.
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Figure 4. Heat map clustering in volatile flavor compounds of kelp samples.

4. Conclusions

In this study, we studied the effect of microbial fermentation on the odor of kelp. Forty-four volatile compounds in unfermented kelp were detected by GC-MS. The most abundant substances were aldehydes, followed by alkanes, then alcohols and ketones. OAV results showed that unsaturated aldehydes and ketones such as 1-octen-3-one, (E,Z)-2,6-nonadienal, (E,E)-2,4-decadienal and (E)-2-nonenal, were the main contributors to the fishy odor of kelp. They contain α,β-unsaturated carbonyl structures, often with extremely low odor thresholds and unpleasant smells. After microbial fermentation, the odor profile changed significantly, which can be directly seen from the GC-IMS fingerprint. Yeast *S. cerevisiae* SK1.008 had a critical impact; the VOCs were diminished to 34, 1-octen-3-one had vanished and the levels of aldehydes had a steep drop, whereas *L. plantarum* FS7 and *P. pentosaceus* CICC 21862 do not have such a strong deodorization performance. The different degrees of reduction in fishy odor may be related to the effect of enone reductase in different microorganisms in catalyzing the reduction of unsaturated bonds. This demonstrates that the strategy of microbial fermentation can influence the odor...
profile of kelp and can even diminish the fishy odor and make strides in the worthiness. The combined use of GC-MS and GC-IMS maximizes the results by combining accurate analytical results with intuitive visualization. Further research should focus on sensory-directed flavor analysis to clarify the flavor of kelp more thoroughly and comprehensively. At the same time, the study of the degradation pathways of fishy-odor compounds by *S. cerevisiae* are also important.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/foods10112532/s1, Figure S1: Gas chromatography profiles of different kelp samples. UF: unfermented kelp; YF: yeast *Saccharomyces cerevisiae* fermented kelp; PF: *Pediococcus pentosaceus* SK1.008 fermented kelp; LF: *Lactobacillus plantarum* FSB7 fermented kelp.

**Author Contributions:** Conceptualization, methodology, software, data curation, writing—original draft preparation, W.Z.; writing—review and editing, formal analysis, J.C.; investigation, resources, F.Z. and T.Z.; writing—review and editing, supervision, project administration, funding acquisition, B.J. All authors have read and agreed to the published version of the manuscript.

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