Characterization of odor components of Pixian Douban (broad bean paste) by aroma extract dilute analysis and odor activity values

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ABSTRACT
The odor-active compounds of Pixian Douban (PXDB) were identified by means of gas chromatography-olfactometry (GC-O) coupled with mass spectrometry (MS), and then the key odor components were figured out by odor activity values (OAVs) and aroma extract dilution analysis (AEDA) using three different methods. The 21 odor active areas were detected with flavor dilution (FD) factor ranging from 2 to 16, with 3-(methylthio) propionaldehyde, 2,5-dimethyl pyrazine, 4-ethyl-2-methoxyphenol, and 2-ethylphenol yielding the highest FD factors. These odorants were then quantitated by application of external standard under selected ion monitoring (SIM) mode of MS, and their OAVs were calculated based on the odor thresholds. The highest OAV was obtained for 3-methylbutanal (malty, 614.89), followed by phenylacetaldehyde (honey-like, sweet, 94.34), four compounds obtained OAVs ≥ 10, and other eight compounds obtained OAVs between 1 and 10. All of them likely contributed to the aroma of PXDB. A reconstitution model containing 14 odorants (OAVs ≥1 & FD>5) was able to mimic the aroma style of the original PXDB. In addition, the application of omission test was used to investigate the effects of the key odor compounds on the whole odor profile of PXDB, and 11 odorants detected were significantly different among the samples.

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Introduction

East Asians have been good at making bean pastes since ancient times and have benefited from the unique flavor and health functions of these pastes. Different cultures and climates, people’s tastes, and production methods make different bean pastes unique in style and characteristics. Pixian broad bean paste, also called Pixian Douban (PXDB), is a famous Chinese condiment, named after the town of Pixian in southwest China’s Szechuan province. The Pidu District, located in a basin with a subtropical humid climate, provides good climate, humidity, sunlight and water quality for the fermentation of PXDB. The microbial community growing in this excellent environment may promote the creation of its unique flavor. PXDB is a spicy and salty fermented paste produced from blanched broad bean, red chili pepper, salt, and Aspergillus oryzae. It usually takes one to three or eight years to ripen and get its characteristic appearance (reddish-brown luster), palatable taste, and strong savory aroma. It is widely used as a key ingredient in Szechuan cuisines, and often referred to as the “soul of Szechuan cuisine”.

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The interest in fermented bean food products has gained considerable attention due to the product’s characteristic flavors and the potential nutritional relevance associated with their consumption. The evidence of this interest is supported by a number of extensive studies reporting on the volatile compounds of fermented bean foods. Previous work revealed 25 key aroma compounds from five different Japanese soy sauces by aroma extract dilution analysis (AEDA), among them 3-(methylthio) propionaldehyde and 3-hydroxy-4,5-dimethyl-2(5H)-furanone with high flavor dilution (FD) factor were thought to play a critical role in the soy sauce products. In a research of Tempeh, 2-acetyl-1-pyrroline, dimethyl trisulfide, 2-ethyl-3,5-dimethyl pyrazine, methional, and 2-methylpropanal and (E, E)-2,4-decadienal were found to be the main aroma compounds. Previous work also found that in Natto, pyrazines, and sulfur-containing compounds worked as critical compositions to the characteristic aroma of Natto. They also reported that the pyrazines and sulfur-containing compounds could mask the beany odor. H. Y. Chung, P. K. Fung, and J. S. Kim identified 68 volatile components of commercial plain sufu extracts and found esters, alcohols, and acids were the majority classes of these compounds. Also, the research found that the key volatiles that related to differentiation of traditional and commercial Doenjang were 2-pentylfuran, 4-ethylphenol, dihydro-5-methyl-2(3H)-furanone, butanoic acid, pyrazines, esters, maltol, dimethyl disulfide, 2- and 3-methylbutanal, hexanal, 4-vinylphenol, and ethanol.

Most previous studies on PXDB have focused on processes and microorganism with an extremely limited number of investigations geared towards the analysis of volatile compounds. Moreover, most studies on the flavor compounds in PXDB only focus on the detection of volatile flavor substances mainly by gas chromatography-mass spectrometry (GC-MS), with the application of quantitative analysis and olfactometry technology being rarely involved. One hundred and nine volatiles were identified by simultaneous distillation-extraction (SDE) and found that esters, alcohols, aldehydes, and ketones were the main volatiles in three grade samples of PXDB. Two PXDB from different factories extracted by SDE were analyzed and indicated there were some differences in the number of volatiles between the two products because of the different fermentation process. Our preliminary investigations suggested that heat has actively impacted on these volatiles during the fermentation of PXDB. However, the extent to which these volatiles in the PXDB are quantitatively affected by these conditions is yet to be studied to the best of our knowledge. The 81 volatile compounds of PXDB were found by a combination of solid-phase microextraction (SPME) and GC-MS, benzaldehyde, 2-pentylfuran, 3,7-dimethyl-1,3,6-octanetriene and tetramethylpyrazine were high in relative area. After that, a study identified 82 volatile compounds from the fermentation of 0 to 3 years in the PXDB and reported that high concentrations of ethyl palmitate, ethyl linoleate, ethyl oleate, 4-ethylphenol, 4-ethylguaiaicol, and phenylethyl alcohol might contribute most to the overall aroma of PXDB. However, in the preliminary studies, qualitative analysis was applied only by MS of databases and quantitative analysis was performed with semi quantification on the amounts of volatiles using the respective peak areas, which very likely leads to unreliable results.

In addition, no detailed research is known to have been conducted on the identification of the aroma-active compounds of PXDB. As we all know, only a small part of the volatile compounds contributes to the overall odor of one food. To identify the key odorants of a certain food accurately we need to use both analytical and sensorial investigations. Gas chromatography-olfactometry (GC-O) technology is an important means of determining the aroma contribution of volatile flavor compounds. The aroma extract dilution analysis (AEDA) and the odor activity values (OAVs, ratio of concentration to odor threshold) based on quantitative analysis have been widely used in products such as fruits, soy sauce, green tea, and Chinese soybean pastes.

Hence, this study was designed to focus on details about the contribution of single odorant to the overall odor of PXDB to obtain a systematic characterization of the key odor compounds. First, the aroma-active compounds of PXDB were identified by three aroma extract dilution methods suitable for solid-phase microextraction. Next, aroma compounds were quantified using external standard quantitation. Finally, the key odorants were further ensured via OAVs and verified by using recombination and omission experiments.
Materials and methods

Material

The aroma composition used for this study was based on first-level (the most popular kind of PXDB in the market) Juan Cheng Pixian Douban (Juan Cheng Brand is China Time-honored Products whose history dates back 400 years ago) from Sichuan Pixian Douban CO., LTD (Chengdu, China).

Chemicals

1,2-dichlorobenzene (internal standard, IS), C6 ~ C20 n-alkane mixture, 2-methyl-1-propanol, 3-methylbutanal, 2-methylbutyraldehyde, 3-hydroxy-2-butanone, 3-methyl-1-butanol, furfural, ethyl 2-methylbutyrate, 3-methylbutanoic acid, 3-(methylthio)propionaldehyde, 2, 5-dimethyl pyrazine, 5-methyl furfural, benzaldehyde, 2-acetylpyrrole, 1-nonanal, phenylacetaldehyde, 2, 3, 5, 6-tetramethylpyrazine, 2-phenylethanol, 2-ethylphenol, 2-phenylbutanoate, 4-ethyl-2-methoxyphenol were obtained from Sigma–Aldrich (Shanghai, China). Dichloromethane and all other chemicals (AR) were purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China).

Extraction of volatile compounds

Fully ground samples (4.0 g) were mixed with 2 mL saturated brine and transferred into 20 mL headspace vial (Supelco, Inc., Bellefonte, PA). Ten μL of 1, 2-dichlorobenzene (500 μg/L in methanol) was added to each sample as an internal standard. The extraction of volatile compounds in PXDB was performed by SPME triplus automated sampler (75 μm CAR/PDMS fiber) (Supelco, Inc., Bellefonte, PA). After constant temperature equilibration at 55°C for 30 min, the CAR/PDMS (75 μm) was used to extraction at 55°C for 40 min. Each sample was extracted in triplicate. Shimadzu GC-MS system (Shimadzu QP2010 Plus, Shimadzu Co., JP) was employed for the analysis of the volatiles. Volatiles were separated by DB-WAX-UI column (30 m, 0.32 mm i.d., 0.5 μm film thickness, Agilent Co., USA). Helium was used as the carrier gas (flow rate of 1.0 mL/min). The injector temperature was at 250 °C and split ratio of 10: 1. The oven temperature was set at 40 °C and held for 3 min, raised 150°C at a rate of 7°C/min, then raised to 185 °C at a rate of 3°C/min, holding for 10 min, and held at 220°C for 10 min. Choose 70 eV MS electron ionization (EI) mode and set scanning range from 35 to 350 amu. The fiber was desorbed for 5 min at 250°C. The column was aged at 220°C for 20 min to remove residual impurities between two samples.

GC-O analysis was conducted using ODP 3 (Gerstel, Germany) with a humid air equipment. The column flow was separated by a 1:1 ratio between the MS and the sniffing port. Olfactometry analysis was carried out by four experienced assessors. All analyses were performed in triplicate. The retention time and odor intensity were recorded using an acoustic recorder. The intensity of the odor was determined by a sensory evaluation, with a total of four levels: 1 for weaker, 2 for weak, 3 for medium, 4 for strong.

SPME-AEDA

The original aroma concentrates extracted by a simultaneous distillation extraction (SDE) or a liquid-liquid extraction (LLE) were step-wisely diluted. When SPME was used, there was no liquid extract because the analyte remained on the fibers. Therefore, the usual AEDA cannot be applied. In this study, the original aroma concentrate extracted by SPME was step-wisely diluted by three methods [18–20]:

(i) Diluted by different split ratios, follow the scale of 2: 1, 4: 1, 8: 1, 16: 1, 32: 1, to 64:1.
(ii) Diluted by different sample weight, which varied from 4 g, 2 g, 1 g, 0.5 g, 0.25 g to 0.125 g.
(iii) Diluted by different time of extraction, follow the scale of 40 min, 20 min, 10 min, 5 min, 2.5 min to 1.25 min.

The FD factor is expressed as the maximum dilution factor: 1 to 6.

**Identification and quantification analysis**

Identification was based on reference standards by retention indexes (RIs) and mass spectra matching in standard NIST 12 libraries and aromatic characteristics and odor-active compounds established by sniffing and comparison to reference standard compounds. The retention index was calculated using the C6-C20 normal paraffin series (Supelco) under the same chromatographic conditions. Sometimes odor thresholds were a good method for determining the true contributors when similar aromatic properties cause the co-elution of several compounds.

Semi-quantitative measurements were made with 1, 2-dichlorobenzene. Four milliliters of dichloromethane containing different concentrations of volatile standards, 5 μL of 1,2-dichlorobenzene (internal standard, IS) and 10 mg/L standard solution was placed in a 20 mL vial. SPME and the GC-MS conditions are set as described above. The aromatic compound (FD $\geq$ 5) was quantified by selective ion monitoring (SIM) mass spectrometry. The standard curve for individual odorant was created by plotting the responded ratio of target compounds and corresponding ISs against the concentration ratio.$^{[21]}$ The ion monitored of IS in the SIM run was m/z 146. The concentrations were calculated in µg/kg.

**Sensory evaluation by reconstitution and omission tests**

**Aroma profile tests**

The sample (20 g) was placed in an odorless glass with a lid and evaluated by a sensory panel. A descriptive profile test was conducted by 10 assessors (5 males and 5 females), they were from GC-O analysis and were laboratory staff study on PXDB. Seven qualities were defined as the most appropriate odor description for the overall odor of PXDB (Table 3). The assessor was required to assess the odor intensity of these attributes on a 10-point scale. The sensory score of the aroma intensity was calculated from the average of 10 panelists and finally plotted in the radar. All the tests were conducted in a sensory analysis test room with eight evaluation cubicles at 20 ± 1°C.

**Recombination and omission tests**

The Recombination solution was prepared according to the concentration value obtained by quantitative analysis, and the aroma compounds were added to the aroma-removed PXDB-salt solution (18%) and then sufficiently mixed and standing for 24 h. In triangular tests, models in which one odorant was omitted were evaluated against two samples of the complete model, sensory panels were expected to distinguish the missing compound.

**Results and discussion**

**Identification of odor-active compounds**

A total of 21 odor-active compounds were revealed by GC-O investigation (Fig. 1). To identify the most important aroma-active compounds in PXDB, three AEDA methods were used in this research, and the FD value of each AEDA dilution method was represented by the uniform number 1–6.$^{[22]}$

As can be seen from Fig. 1, the most intense aroma-active compounds from PXDB were located by AEDA, and the FD factors ranged between 1 and 16. The 21 odor-active compounds were analyzed based on their flavor characteristics and categorized according to the resultant distinct aroma that they exhibited. Among them, compound 9 (coconut-like, soy sauce-like), compound 10 (caramel-like),
Figure 1. Flavour dilution chromatogram indicating the key odourants in the gas chromatogram of the volatile fraction isolated from PXDB (FD factor 1, 2, 3 represent diluted by different split ratios, different sample weight, different extract time, respectively).
compound 18 (popcorn-like), compound 20 (vanilla-like), and compound 21 (gammon-like, smoky) showed the highest FD factor in three methods, followed by compound 13 (foxy), and compound 17 (beeswax-like, honey-like) with a total FD factor of 11. Other aroma presented were compound 16 (citrus-like, flowery) and compound 2 (fatty, green) with a total FD factor of 10. Another honey-like-smelling compound (15) and a component with a nut-like odor (6) were also perceived.

The structure of odorants detected during AEDA was obtained by comparing the respective odor profiles, the intensity at the sniffer port and the retention index on the capillary columns of two different polarities by reference to the data of the volatiles in the internal database. By adopting this method, we can identify the odorants with the highest FD factor being identified as 3-methylthiopropionaldehyde (compound 9), 2, 5-dimethyl pyrazine (compound 10), 4-ethylphenol (compound 18), and 4-ethyl-2-methoxyphenol (compound 20).

3-methylthiopropionaldehyde is a sulfur-containing compound with a mellow sauce flavor and low threshold can be clearly smelled by GC-O and has also been previously detected in soy sauce.[23] 4-ethyl-2-methoxyphenol was identified as one of the main components of the aroma of PXDB.[24] Moreover, 2-pyrole (compound 13), 2-phenylethanol (compound 17), benzaldehyde (compound 12), tetramethylpyrazine (compound 16), and phenylacetaldehyde (compound 15) were detected with high FD factors. Phenylacetaldehyde has also shown a strong intensity and considered as the aroma-active compound in a Chinese natural fermented soybean paste sample.[3] Phenethyl alcohol (flowery-fruity) is an important aroma in many foods, such as gooseberry fruit[15] and tomato.[25] The other compounds with low FD factor were considered with low importance, even no contribution to the flavor of PXDB.

**Quantitation of important odorants and calculation of odor activity values (OAVs)**

Having utilized the AEDA as a ranking method, we got the first set of information on which aroma compounds would most likely contribute to the overall aroma of PXDB. However, the contributions of aroma compounds in PXDB were not just determined by ADEA, but also their interactions with the matrix. The volatility of a compound and its concentration in the headspace above the food were affected by the matrix.[26] For this purpose, the OAVs concept was further applied to identify the odorants in PXDB. Among the 21 odorants quantified, 16 compounds that showed high FD factors (>5) were quantified by means of the external standard method, and the remaining five odor active compounds are quantified by internal standards and then their OAVs were calculated.

The quantitation results shown in Table 1 revealed that furfural (4300.09 µg/kg), 2, 5-dimethyl pyrazine (2821.98 µg/kg), 3-methyl-1-butanol (2167.13 µg/kg), and tetramethylpyrazine (1228.20 µg/kg) were the most abundant odorants in PXDB. Followed by 2-phenylethanol (1047.73 µg/kg) and 3-methylbutanal (1007.46 µg/kg). Among them, 2-phenylethanol, 3-methyl-1-butanol, and furfural were also calculated as the most abundant compounds in the previous research.[10]

Furfural gave sweet and almond odor. It was produced by microbial fermentation of five carbon sugars. Y. Feng declared that although furfural has a high threshold, its role in soy sauce should not be underestimated.[2] It can interact with other flavor substances and has the effect of enhancing the taste of soy sauce. It is worth noting that pyrazines were high in concentration. It was reported that pyrazine is formed by the activities of microorganisms. The process of fermentation, high-temperature production, and grain stacking of soy sauce-flavored liquor before fermentation could increase the generation of pyrazines.[27] Alcohols are also a large group of substances, which produced by the metabolism of reducing sugars by microorganisms.

Ten compounds were detected in concentrations between 100 and 700 µg/kg, including isovaleraldehyde (676.38 µg/kg), 2-acetylpyrrole (601.10 µg/kg), 4-ethyl-2-methoxyphenol (516.26 µg/kg), benzaldehyde (497.48 µg/kg), and Phenylacetaldehyde (377.34 µg/kg). On the other hand, the concentrations of some aroma compounds were below 100 µg/kg, including 5-methyl furfural and 3-(methylthio) propionaldehyde. Ethyl 2-methylbutyrate and 1-nonanal showed lower concentrations below 10 µg/kg.
According to the concept of OAV, if the odor exceeds its threshold, the scent should contribute to the overall aroma. Quantitative results were used to calculate their OAVs. As shown in Table 2, except two volatiles having no OAVs as no threshold was available, the OAVs data revealed 14 among 21 aroma-active compounds with concentrations above their odor thresholds. Among them, the highest OAV was observed for 3-methylbutanal (OAV = 614.89), which was a key aroma compound in PXDB with a malty aroma. Phenylacetaldehyde with honey-like, sweet aromas also exhibited higher OAV and thus became the main substance that provided sweetness in PXDB. 3-Methylbutanal and phenylacetaldehyde were known as main soy sauce volatiles and were found to come from free amino acids formed during soy fermentation.\(^{[26]}\)

The results further revealed four other compounds which presented OAVs >10, including, 3-(methylthio)-propionaldehyde (47.43) with a cooked potato and soy sauce-like aroma, 2-acetylpyrrole (31.64) with roasted potatoes aroma, 2-methylbutanal (malty, 27.11), and 4-ethyl-2-methoxyphenol (10.33) with smoky, warm and herb-like aroma. These compounds appeared to contribute significantly to the aroma of PXDB and were considered as its dominant odor impression. 3-(methylthio)-propionaldehyde was also found in other fermented products, such as Korean soy sauce, high-salt soy sauce, and yeast extraction.\(^{[26]}\)

However, not all Strecker aldehydes in foods are welcomed. Studies have shown that the content of 3-(methylthio)-propionaldehyde and phenylacetaldehyde in beer is negatively correlated with its quality. The increase in beer storage period has been one of the sources of beer aging flavor.\(^{[33]}\) 2-acetylpyrrole exhibited high odor impressions typical for roasted potatoes aroma in PXDB. But the intensity of 2-acetylpyrrole of wort was very low in the fermented beverage.\(^{[34]}\) It was reported to be a major odor active compound in a new wort beverage fermented with shiitake mushrooms\(^{[35]}\) 4-ethyl-2-methoxyphenol gave smoke, bacon and wood aroma to the PXDB, and was detected in soy sauce, vinegar, sweet sauce, and other food products. Among them, 4-ethyl-2-methoxyphenol is an important contributor to the attractive flavor of Japanese and Korean soy sauce, and it has been reported to be related to the metabolic activity of yeast.\(^{[36]}\)

OAVs ≥1 were also obtained for 3-methyl-1-butanol (alcoholic, fruit, sour), 2-ethylphenol (green, raw pepper-like), 2-phenylethanol (rose-like, flowery), and soy sauce-like 2, 3, 5, 6-tetramethylpyrazine. They

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**Table 1. Quantitative analysis of 21 odourants in PXDB**

| No. | aroma compound                  | quantifying ion (m/z) | slope | Intercept | R²      | concentrations (µg/kg) | RSD⁷ | quantitative methods⁶ |
|-----|---------------------------------|-----------------------|-------|-----------|---------|-----------------------|------|----------------------|
| 1   | 2-Methyl-1-propanol             | 43(42,41)             |       |           |         | 140.02                | IS   | A           |
| 2   | 3-Methylbutanal                 | 44 (43, 58)           | 127.1 | −9.9      | 0.998   | 676.38                | 8%   | IS, ES   |
| 3   | 2-Methylbutyraldehyde           | 57 (58, 41)           | 880.1 | 169.6     | 0.999   | 119.29                | 3%   | IS, ES   |
| 4   | 3-Hydroxy-2-butanone            | 45(88, 43)            | 243.7 | 18.3      | 0.992   | 141.82                | 7%   | IS, ES   |
| 5   | 3-Methyl-1-butanol              | 55 (42, 43)           | 188.5 | −24.6     | 0.994   | 2167.13               | 6%   | IS, ES   |
| 6   | Furfural                        | 96 (95, 39)           | 121.4 | −1.3      | 0.992   | 4300.09               | 3%   | IS, ES   |
| 7   | Ethyl 2-methylbutyrate          | 57(102, 41)           | 107.1 | 11.3      | 0.991   | 2.08                  | 8%   | IS, ES   |
| 8   | 3-Methylbutanoic acid           | 60 (41, 43)           |       |           |         | 1007.46               | IS   | A           |
| 9   | 3-Methylthiopropionaldehyde     | 48 (104, 47)          | 166.0 | 6.1       | 0.998   | 66.41                 | 2%   | IS, ES   |
| 10  | 2,5-Dimethyl pyrazine           | 108 (42,40)           | 131.3 | −1.9      | 0.996   | 2821.98               | 5%   | IS, ES   |
| 11  | 5-Methyl furfural               | 110 (109, 53)         |       |           |         | 32.13                 | IS   | A           |
| 12  | Benzaldehyde                    | 105 (106, 77)         | 102.4 | −2.8      | 0.994   | 497.48                | 1%   | IS, ES   |
| 13  | 2-Acetylpyrrole                 | 94 (103, 66)          | 138.5 | −6.2      | 0.992   | 601.10                | 4%   | IS, ES   |
| 14  | 1-Nonanal                       | 57 (98, 43)           | 170.9 | −0.1      | 0.991   | 6.09                  | 10%  | IS, ES   |
| 15  | Phenylacetaldehyde              | 91 (92, 120)          | 46.4  | 8.8       | 0.994   | 377.36                | 2%   | IS, ES   |
| 16  | Tetramethylpyrazine              | 136 (54, 42)          | 120.5 | 5.0       | 0.998   | 1228.2                | 1%   | IS, ES   |
| 17  | 2-phenylethanol                 | 91 (92, 120)          | 49.1  | −0.2      | 0.997   | 1047.73               | 2%   | IS, ES   |
| 18  | 4-Ethylphenol                   | 107 (122, 77)         | 124.2 | 14.6      | 0.995   | 286.97                | 2%   | IS, ES   |
| 19  | Ethyl phenylacetate             | 93 (164, 65)          |       |           |         | 253.84                | IS   | A           |
| 20  | 4-Ethyl-2-methoxyphenol         | 137 (152, 39)         | 71.8  | −6.4      | 0.993   | 516.26                | 1%   | IS, ES   |
| 21  | Unknown⁴                        | -                     | -     | -         | -       | 143.92                | IS   | A           |

⁶Quantitation based on IS (internal standard method) and/or ES (external standard method). ⁷RSD, relative standard deviation of the average concentration. ⁸-: no corresponding values obtained due to the only use of IS. ⁹Unknown: compound failed to identification.
were, therefore, also suggested to contribute significantly to the overall aroma of PXDB. Alcohol, green, and flowery aroma from 3-methyl-1-butanol, 2-ethylphenol, and 2-phenylethanol contribute to rich, complex and concentrated, and layered aromas of PXDB. 2, 3, 5, 6-tetramethylpyrazine was an important compound with cooked potato and soy sauce-like aroma. Bacteria and yeast enter the fermentation tank from the environment in the post-fermentation stage. During the process, tetramethylpyrazine was produced by bacillus subtilis from two molecules of acetoin (3-hydroxy-2-butanone) and two molecules of ammonia. Wu reported that in the brewing of liquor, tetramethylpyrazine produced in the stage of bacterial koji and was one of the main compounds in wine.

Sensory analysis by aroma recombination and omission experiments

Recombination
Validation of quantitative data based on reconstitution experiments is important to determine whether certain aromas were key aroma compounds in the mixed food flavor. The model mixture contained all compounds with OAV ≥1 given in Table 2. A sensory panel compared the aroma of reconstitution and the authentic PXDB. Odor qualities were selected by pre-experiment and were divided into seven groups according to the aroma style (Table 3).

Flavor reconstruction experiments are an effective tool to verify the correctness of quantitative data. The aroma characteristics of the authentic samples and the recombinants were highly similar, with an overall similarity score of 8.6. All aroma qualities of cooked potato-like, grassy/green, sour, honey-like, flowery, musty, and malty were similar in both the authentic sample and the aroma-recombined sample (Fig. 2). Among them, the soy sauce-like aroma quality was rated with

| NO. | Compounds                        | Odour descriptiona | Retention index | Thresholds (µg/Kg)b | OAVs  |
|-----|----------------------------------|--------------------|-----------------|---------------------|-------|
| 1   | 2-Methyl-1-propanol              | Slightly sour      | 597             | 7000[28]            | 0.02  |
| 2   | 3-Methylbutanal                  | Malty, almond      | 643             | 1.1[27]             | 614.89|
| 3   | 2-Methylbutanol                  | Reagent-like, almond | 650    | 4.4[27]             | 27.11 |
| 4   | 3-Hydroxy-2-butanol              | Sweet              | 717             | 800[29]             | 0.18  |
| 5   | 3-Methyl-1-butanol               | Fruit, slightly sour | 811    | 220[20]            | 9.85  |
| 6   | Furfural                         | Nuts               | 831             | 3000[20]            | 1.43  |
| 7   | Ethyl-2-methylbutyrate           | Floral             | 841             | 1[31]               | 2.08  |
| 8   | 3-Methylbutanoic acid            | Sour, musty        | 845             | 1200[27]            | 0.84  |
| 9   | 3-(Methylthio)-propionaldehyde  | Sauce, cooked potato | 858   | 1.4[27]             | 47.43 |
| 10  | 2,5-Dimethyl pyrazine            | Cooked peanut      | 932             | 2600[20]            | 1.09  |
| 11  | 5-Methyl furfural                | Musty              | 957             | -                   | -     |
| 12  | Benzaldehyde                     | Roasted nuts       | 982             | 350[31]             | 1.42  |
| 13  | 2-Acetylpyrrole                  | Cooked potato      | 1035            | 19[28]              | 31.64 |
| 14  | 1-Nonanal                        | Floral             | 1108            | 8[27]               | 0.76  |
| 15  | Phenylacetaldelyde               | Honey-like         | 1081            | 1644                | 94.34 |
| 16  | Tetramethylpyrazine              | Soy sauce-like     | 1121            | 1000[20]            | 1.23  |
| 17  | 2-phenylethanol                  | Rose-like, floral  | 1135            | 211[27]             | 4.97  |
| 18  | 2-Ethylphenol                    | Raw pepper, green  | 1168            | 40[27]              | 7.17  |
| 19  | 2-phenylbutanoate                | Floral             | 1229            | 650[32]             | 0.39  |
| 20  | 4-Ethyl-2-methoxyphenol          | Sweet, warm, spicy, herb-like | 1299 | 50[34]              | 10.33 |
| 21  | Unknown                         | Grass, green       | 1403            | -                   | -     |

aOdour description: based on (I) the University of Florida Citrus Flavour database (http://www.crec.ifas.ufl.edu/rouseff/#) and (II) Flavour net (http://www.flavournet.org/flavournet.html). bThresholds were taken from the references about fermented sauce with same matrix. cThresholds on http://www.leffingwell.com/odourthre.htm. d-: failed to find the threshold. eUnknown, not identified due to trace amounts.

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Table 2. Odour activity values (OAVs) of the odour-active compounds identified in PXDB
the highest score by the sensory panelists. Besides, floral, musty, grassy/green, and honey-like aromas were perceived to have the nearly same intensity. However, the spicy and sour group had a lower intensity in the aroma-recombined sample. This may be explained by the nonvolatile compounds’ influence on the release of the odorants from the matrix.\cite{39}

In total, these data proved that the key aroma of PXDB was successfully identified and quantified.

Omission experiments were performed to confirm the association of certain aromatic compounds with the aroma quality of PXDB, and the results are displayed on Tables 3 and 4. The assessors need to assess the degree of similarity between the intact model and the incomplete models with omitted compounds. Eleven key odorants were identified from the 14 aroma compounds (Table 2). The data showed that the panels could identify the omission of the aroma of significance. The highest significant odor aroma included 3-methylthiopropanal, benzaldehyde, 2, 3, 5, 6-tetramethylpyrazine, 2, 5-dimethyl-1-pyrrole, and phenylacetaldehyde in accordance with previous studies where four of these volatile compounds were considered as the key aroma component for model soy sauce.\cite{22}

This result reflected the important role of these cooked potato, malty, soy sauce-like, honey-like smelling compounds played in the overall PXDB

| Table 3. Grouping of PXDB aroma description |
|---|---|---|---|
| No. | type | odour profile | odourant |
| 1 | Soy sauce-like | Soy sauce, cooked potato, malty | 3-Methylthiopropanal, furfural, benzaldehyde, 2-acetylpyrrole, 2,5-dimethylpyrazine, 2,3,5,6-tetramethylpyrazine |
| 2 | Alcoholic | Floral, fruits | 3-Methyl-1-butanol, 2-phenylethanol, ethyl 2-methylbutyrate |
| 3 | Sweet | Sweet, honey-like | Phenylacetaldehyde |
| 4 | Grass | Grassy, green, smoky, herb-like | 2-Ethylphenol, 4-ethyl-2-methoxyphenol |
| 5 | Spicy | Spicy, pungent | 4-Ethyl-2-methoxyphenol, 2-ethylphenol |
| 6 | Sour | Sour | 3-Methylbutanoic acid |
| 7 | Musty | Musty | 3-Methylbutanoic acid |

Figure 2. The sensory score of PXDB (solid line indicates authentic PXDB, and broken line indicates recombination samples).
aroma. Thus, cooked potato and soy sauce-like notes were often used as an aroma standard for the evaluation of flavor quality of PXDB.

Besides, 2-acetylpyrrole, 2-ethylphenol, 2-phenylethanol, furfural, 4-ethyl-2-methoxyphenol, and 3-methylbutanal also showed a significance level between omission model and PXDB. Three of them exhibited a soy sauce-like type aroma, which strengthened the main soy sauce-like aroma of PXDB. Equally, floral from 2-phenylethanol, 2-ethylphenol with grassy/green aroma, and smoky/herb-like aroma from 4-ethyl-2-methoxyphenol also made up the unique aroma of PXDB. The omission of 3-methyl-1-butanol, 2-methylbutyaldehyde, and ethyl 2-methylbutyrate could be recognized only 7 times of 24 tests, thus reflecting that these compounds have no significant effect on the overall aroma.

**Conclusion**

In summary, the aroma-active compounds in PXDB were identified by SPME-GC-O/MS and revealed 21 aroma-active areas. The AEDA and OAVs analysis were used to analyze the key aroma components of PXDB. 3-(Methylthio) propionaldehyde, 4-ethyl-2-methoxyphenol, 2, 5-dimethyl pyrazine, and 2-ethylphenol recorded the highest FD. Among 17 odorants with FD≥5, 14 of them were found at concentrations above their odor thresholds. The highest OAV was observed for 3-methyl-1-butanol, followed by phenylacetaldehyde. Aroma recombination and omission experiments were conducted by a trained sensory panel and further confirmed the PXDB aroma can be closely mimicked using only 11 aroma compounds in their natural concentrations. And 3-(methylthio) propionaldehyde, benzaldehyde, 2, 3, 5, 6-tetramethylpyrazine, phenylacetaldehyde, 2,5-dimethylpyrazine, 2-acetylpyrrole, 2-phenylethanol, 2-ethylphenol, furfural, 4-ethyl-2-methoxyphenol, and 3-methylbutanal were distinguished as the most predominant compounds in PXDB, constituting a unique and complete PXDB flavor. This study, for the first time, described a comprehensive profile of the aroma-active compounds in PXDB, further work should be directed to the effects of PXDB non-volatiles on aroma quality and the effects of microbial action on PXDB flavor formation to elucidate the mechanism of flavor compounds and provide valuable information to the industry.

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