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Enhancement of saltiness perception by odorants selected from Chinese soy sauce: A Gas Chromatography/Olfactometry-Associated Taste study

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Running title: identification of salty-associated odorants in soy sauce

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ABSTRACT

Odor-taste interaction has become a popular salt reduction method. In this study, the odorants associated with saltiness in soy sauce were selected by gas chromatography/olfactometry-associated taste (GC/O-AT), and their ability to induce saltiness/umami enhancement was verified by sensory evaluation. A total of 30 taste-associated odorants were perceived, including 5 saltiness-associated and 2 umami-associated odorants. Among them, 3-(methylthio)propanal, 1-octen-3-ol, 3-(methylthio)-1-propanol, and 2,5-dimethylpyrazine could significantly enhance saltiness of 0.3% NaCl solution ($p < 0.05$). Furthermore, 3-(methylthio)propanal, maltol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF), dimethyl trisulfide, 3-(methylthio)-1-propanol and 1-octen-3-ol could also enhance the umami taste in 0.3% monosodium glutamate solution. Compared with zero or strong-salt-content (0.8%) solution, the saltiness of weak-salt-content (0.3%) was enhanced significantly by adding the odorant. These results suggest that salty food is an efficient source for selecting saltiness-enhancing odorants, which could be used to compensate NaCl reduction in food.

Keywords
Odor-induced taste, soy sauce, gas chromatography/olfactometry-associated taste, 3-(methylthio)propanal, salty taste enhancement
1. Introduction

The overconsumption of salt is a global health issue and widely recognized for contributing to cardiovascular diseases, stroke, and renal diseases (He & MacGregor, 2009). Nowadays, the average consumption is 10.5 g of salt per day in China while it is 7–13 g in Europe (Chinese Society of Nutrition, 2018; Kloss, Meyer, Graeve, & Vetter, 2015), both higher than the 5 g recommended by the World Health Organization (2012). Therefore, "China Industrial Salt Reduction Plan" recommended the reduction of the per capita salt intake by 20% before 2030 (Chinese Society of Nutrition, 2018).

Various methods have been proposed to reduce the salt content in food. For example, there is a widespread concern about salt substitutes, i.e. KCl, which allow reduction of the NaCl content by up to 50% in meat products (Desmond, 2006). However, KCl has negative sensory characteristics, such as strong bitterness, weak saltiness, and metallic properties (Desmond, 2006) and is not suitable for nephritic patients, who have to control potassium intake strictly (Geleijnse, Kok, & Grobbee, 2003). Therefore, some researchers have turned to non-mineral salt replacers, such as monosodium glutamate, and naturally brewed soy sauce (Kremer, Mojet, & Shimojo, 2009; McGough, Sato, Rankin, & Sindelar, 2012). Moreover, other methods could help to reduce sodium intake, such as size and distribution of the salt (Moncada, Astete, Sabliov, Olson, Boeneke, & Aryana, 2015). Recently, odor-induced saltiness enhancement (OISE), an approach of reducing salt based on odor–taste interactions, has attracted increasing attention (Lawrence, Salles, Septier, Busch, & Thomas-Danguin, 2009; Nasri, Beno,
For instance, the specific salty-related odors (such as bacon, anchovy, or sardine odor) applied by the retronasal route could enhance the saltiness at low or medium NaCl concentration (Lawrence et al., 2009; Nasri et al., 2011). Moreover, ham and sardine odors, but not carrot, were shown to enhance saltiness in solid foods, such as cheese (Syarifuddin, Septier, Salles, & Thomas-Danguin, 2016), showing the congruency between odor and taste.

In Asian countries, soy sauce is a popular fermented seasoning due to its salty, umami and rich flavor (Feng, Su, et al., 2015; Feng, Cai, Su, Zhao, Wang, & Zhao, 2014). According to the previous studies, soy sauce flavor could enhance salty taste perception not only in NaCl solution (Lawrence et al., 2009; Onuma, Maruyama, & Sakai, 2018), but also in actual food, such as beef soup (Lee, Lee, & Kim, 2015) and roasted peanuts (Chokumnoporn, Sriwattana, & Prinyawiwatkul, 2016). However, the odorants of soy sauce that could contribute to saltiness enhancement was not studied. Numerous aroma-active compounds (>50) were detected in soy sauce (Feng, et al., 2014); thus it would be time consuming to verify each odorant by sensory evaluation. A novel method, gas chromatography/olfactometry-associated taste (GC/O-AT) (Barba, Beno, Guichard, & Thomas-Danguin, 2018), was applied to select odorants using 4 taste descriptors (sweet, salty, bitter, sour) from a commercial multi-fruit juice, and then odorants associated with sweet were applied to enhance sweetness perception in a sensory experiment.

Therefore, the aims of this study were to select the odorants related to taste (sour, sweet, bitter, salty and umami) from soy sauce by GC/O-AT, and then to verify the
saltiness/umami enhancement abilities of those odorants associated with salty and umami by performing sensory experiments.

2. Materials and methods

2.1. Soy sauce samples

A bottle of Chinese commercial soy sauce (high-salt liquid-state fermentation soy sauce, Haitian Co., Foshan, Guangdong) was purchased from a local supermarket in Guangzhou, China and stored at 4 °C until use.

2.2. Chemicals

The volatile standards, i.e. phenylacetaldehyde (95%), 3-methylbutanoic acid (99%), 3-(methylthio)propanal (98%), 4-vinylguaiacol (≥ 98%), ethyl isobutyrate (99%), 3-hydroxy-2-methyl-4-pyrone (maltol, 99%), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF, ≥ 99%), 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone (HEMF, mixture of isomers, 96%) and standards of n-alkanes (C6–C33, 99%) were purchased from Sigma-Aldrich (Steinheim, Germany). 3-Methyl-1-butanol (99.8%), ethyl butyrate (≥ 99.5%), 1-octen-3-ol (98%), 2-methylbutanoic acid (98%), 4-ethyl-2-methoxyphenol (98%), 2-phenylethanol (98%), dimethyl trisulfide (98%), 3-(methylthio)-1-propanol (99%) and 2,5-dimethylpyrazine (98%) were purchased from Aladdin (Shanghai, China). In the GC/O-AT and sensory evaluation sessions, nine food-grade volatile compounds, including phenylacetaldehyde (>99%), 3-hydroxy-2-methyl-4-pyrone (>96%), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (>98%), 3-(methylthio)propanal (>98%), 1-octen-3-ol (>96%), 3-(methylthio)-1-propanol (>99%), dimethyl trisulfide (>95%), 2,5-dimethylpyrazine (>99%), and 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone
(>98%), were purchased from Huabao Co. (China). Distilled water was obtained from Watsons Co. (Guangdong, China). Food-grade sodium chloride was purchased from Xiangheng Co. (Hunan, China).

2.3. Liquid–liquid extraction (LLE) of volatile compounds in soy sauce

An aliquot (50 mL) of soy sauce, added with 20 μL internal standard (2-methyl-3-heptanone, 172.4 mg/L in methanol), was extracted by 50 mL dichloromethane three times. After 30 minutes of extraction, the solvent layer was separated by centrifugation (7012 g, 4 °C, 10 min). Then the organic extract was combined, dried over anhydrous sodium sulfate, and kept at –20 °C overnight. The extract was concentrated to 1.5–2.0 mL by Vigreux column (40 cm length; Jingke Instrument Factory, Guangzhou, China), filtered through 0.22 μm organic membrane, and then concentrated to 1 mL by mild nitrogen stream.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis was performed by a Trace GC–MS system analysis, equipped with an Ultra GC, a Trisplus autosampler, and a quadrupole ISQ II MS (Thermo Finnigan, San Jose, CA). A TR-5ms column (60 m × 0.25 mm × 0.25 μm; Thermo Scientific, Waltham, MA) was used. The analytical conditions were as follows: the temperature of the oven was maintained at 40 °C for 6 min, then heated at 4 °C/min to 120 °C, maintained at 120 °C for 2 min, then heated at 10 °C/min to 280 °C with a final isotherm at 280°C for 15 min. Helium was used as carrier gas with a constant flow rate of 2.0 mL/min. The split ratio was 10:1. Mass spectra were obtained in electron impact mode.
(energy voltage 70 eV), with scan rate of 3.00 scans/s and scan range of m/z 33–350. The injection temperature was set as 230 °C, and 280 °C for the ion source temperature. XcaliburTM software Version 2.0 (Thermo Finnigan) was used for analysis of chromatograms and mass spectra. The experimental retention indices (RI) calculated using a series of n-alkanes (C6 to C33) as external references under the same chromatographic conditions. Identification of volatiles was achieved by matching the RI and mass spectra to the spectral library (NIST14 and Wiley Flavors and Fragrances) and authentic compounds.

2.5 Gas chromatography/olfactometry-associated taste (GC/O-AT) analysis

GC/O-AT analysis was performed on a trace GC–MS system equipped with an olfactometric detector (ODP 3; Gerstel, Mülheim an der Ruhr, Germany). The end of the capillary column was connected to a splitter, which divided the effluent into two equal sections, sniffer port and MS detector. Analytical conditions were the same as for GC–MS. During the sniffing experiment, the transfer line was heated to 280 °C to avoid condensation of the effluent; and a humidifier was installed at the olfactory port to avoid dehydration of nasal mucosa. Sniffing time was approximately 30 min. The panelists' aroma description and the perceived intensity were recorded by recording system and buttons (four buttons: 1 = weak, 2 = feeling but not strong, 3 = strong, 4 = extremely strong), respectively.

In experiment 1, GC/O-AT was applied to the selection of odorants associated with 5 taste attributes (sour, sweet, bitter, salty and umami) instead of odor attributes. Ten panelists (4 males and 6 females, aged 19–31) were recruited from South China.
University of Technology. They were trained in two sessions to become familiar with the gas chromatography–mass spectrometry/olfactometry (GC-MS/O) procedure. In session 1, as routine GC–MS/O analysis, the panelists were asked to describe the aroma characteristics and to record the intensity of each odorant by pressing the button; Then in session 2, the panelists were submitted to a second GC run and were asked to describe the eluted odorants with taste attributes (sour, sweet, bitter, salty and umami). If the taste attribute of a stimulus was hard to define immediately, the panelist could freely describe it and then added supplements after GC/O-AT test.

2.6. Sensory analysis

2.6.1. Subjects

Twenty-five panelists (16 females and 9 males, aged 19–35) participated in this study. The panelists were selected according to the following criteria: (1) non-smokers or alcoholics; (2) free from deficits in taste and smell; (3) passed the five basic tastes identification test, and then the discrimination test. Prior to the session, selected panelists had attended a training session to get familiar with the use of the 10 centimeter linear scales. The panelists were asked to refrain from eating spicy food or using any strong-scented personal care products on the day of testing, and not to smoke or to eat at least 1 h before the test.

2.6.2. Sensory evaluation procedure

The sensory evaluation (experiments 2 and 3) was carried out with a trained analytical panel, using a ten-point scale according to the modified methods of Nasri et al. (2011) and Lawrence et al. (2009). All solutions were prepared 3 h prior to the experiment and
stored at 4 °C before use. Samples were served at room temperature (23 ± 2 °C).

In experiment 2, nine food-grade odorants selected in GC-O/AT (experiment 1) were
diluted in distilled water containing 0.3% food-grade NaCl or MSG, to explore their
potential abilities in enhancing saltiness/umami of NaCl/MSG solution. As shown in
Table 2, the concentrations of odorants were selected according to their olfactory
intensity and acceptability in the pre-test. Ten milliliters of each solution were presented
in a 50-mL plastic cup. All samples were coded with random three-digit numbers and
served to the panelists in a randomized complete block design. The aim of the
experiment was not revealed. Prior to tasting samples, panelists were asked to taste
reference solution (0.3% NaCl/MSG solution and 0.8% NaCl/MSG solution, which
were defined as 3 and 10 points). For each sample, the panelists were asked to place the
entire sample in their mouth and swirl the sample around their mouth for 3–5 s, and
then evaluate the perceived taste intensity after expectorating the sample. The panelists
cleaned their mouth with Watson's distilled water. An interval of 90 s was imposed
between each sample. The sensory results were calculated as odor-induced saltiness
enhancement (OISE) and odor-induced umami enhancement (OIUE), namely the
difference between (i) the mean saltiness or umami points for the NaCl/MSG solution
containing an aroma and (ii) the mean saltiness or umami points for the solution
containing only the same amount of NaCl/MSG, respectively.

In experiment 3, the strongest odorant in terms of saltiness enhancement, 3-
(methylthio)propanal selected in experiment 2, was added to solutions with different
salt concentrations (0%, 0.3% and 0.8%), to investigate the influence of salt
concentrations on saltiness enhancement. The sensory analysis was similar to experiment 2, except for the scale identification and sample order. The 0.3%, 0.8% and 1.2% NaCl solutions were defined as 3, 7 and 10 points. The samples were presented from low NaCl concentration to high concentration.

2.7. Data analysis
The OISE and OIUE significance were evaluated by one-sample $t$-test using the statistical package SPSS (version 16.0, Chicago, IL), to assess whether the means were different from 0. The effects were significant when $p < 0.05$.

3. Results and discussion
3.1. Selection of odorants associated with salty and umami taste by GC/O-AT
As shown in Table 1, a total of 30 taste-associated odorants detected in Chinese soy sauce were obtained by gas chromatography–mass spectrometry/olfactometry (GC–MS/O) and GC/O-AT. Among them, 4 odorants were mainly associated with sourness, 15 with sweetness, 4 with bitterness, 5 with saltiness, and 2 with umami. Most of them were the key aroma-active compounds detected in our previous studies (Feng et al., 2015).

Four sour-associated odorants were smelled and identified (Table 1), including 2 acids, 1 alcohol and 1 phenol. Among them, 3-methylbutanoic acid and 2-methylbutanoic acid with “cheese-like” notes were described as “sour” by 87% and 60% panelists. The alcohol (3-methyl-1-butanol) and phenol (4-vinylguaiacol), with "malty, rancid" and
“spicy, burnt” odor respectively, were described as “sour” by less than 50% of panelists. Sweet-associated odorants formed the biggest group, including esters, ketones and phenols mostly. The descriptions of these odorants could be divided into 4 types of aroma, i.e. “fruity”, “caramel-like”, “floral, honey”, and “popcorn, cooked rice”. The detection frequencies of taste description of sweet-associated compounds were all higher than 50%. For bitterness, there were 4 odorants associated to this taste, including 1 unknown compound with “medicine-like” notes, and 3 odorants with “smoky, burnt” notes, i.e. 3-ethyl-2,5-dimethylpyrazine, 2-methoxyphenol, and 4-ethylphenol.

Among the 5 salty-taste-associated odorants, 3 of them were sulfur compounds: 3-(methylthio)propanal (cooked potato), 3-(methylthio)-1-propanol (cooked potato) and dimethyl trisulfide (cooked onion). Particularly, the detection frequency of 3-(methylthio)propanol was up to 100%. In addition, one “unknown” odorant was described as “onion, garlic”. According to the odor description and extremely low threshold of sulfur-containing compounds, the “unknown” compound was presumed to be a sulfur-containing compound.

For umami taste, panelists selected 2 compounds, 1-octen-3-ol (mushroom odor) and 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone (HEMF) (caramel-like odor). Interestingly, HEMF was associated with umami taste, unlike most "caramel-like" compounds, which were associated with sweetness.

3.2. Enhancement of saltiness and umami taste by selected odorants

The influence of 9 odorants, including 4 salty-associated and 2 umami-associated odorants, on the salty and umami taste perceptions was tested. Three additional
odorants associated to sweet taste were selected as controls. In this sensory evaluation study, panelists rated the salty or umami taste intensities of each samples, either NaCl/MSG solution alone or the NaCl/MSG solution with the odorants. OISE and OIUE were calculated for each odorant and the results are reported in Figure 1a and Figure 1b, respectively. As shown in Table 2, the concentrations of these odorants used in this study ranged from 339 μg/L to 3220 μg/L. The concentrations of the odorants were determined through a preliminary sensory test by trained panelists. The selection criteria were that the odorant was perceived with a medium odor intensity when added into the NaCl/MSG solution.

In Figure 1a, the results showed that 3 salty-associated odorants (3-(methylthio)propanal, 3-(methylthio)-1-propanol and 2,5-dimethylpyrazine) and 1 umami-associated odorant, namely 1-octen-3-ol, could enhance the saltiness of 0.3% NaCl solution significantly, while the 3 control sweet-associated odorants (phenylacetaldehyde, maltol, and HDMF) could not enhance the saltiness of the NaCl solution. These observations indicated that the actual taste effects of most selected odorants agreed with their taste association showed in the GC-O/AT experiment. Nevertheless, dimethyl trisulfide (DMTS), associated with salty taste in GC/O-AT, could not enhance the saltiness of 0.3% NaCl solution significantly ($p > 0.05$). This was probably due to the complicated taste description of dimethyl trisulfide in GC/O-AT, including 44% saltiness-associated, 33% umami-associated, and 22% sourness-associated descriptions. Interestingly, the umami-associated odorant 1-octen-3-ol with “mushroom” note could also enhance saltiness perception in 0.3% NaCl solution. It has
been described that umami perception in food could be enhanced by salt (Ugawa & Kurihara, 1994). Indeed, a previous study showed that the positive influences of a dried bonito aroma fraction (DBAF) on saltiness perception were thought to be induced by aroma compounds, especially sulfur-containing compounds, pyrazines, alcohols and phenols, which is in line with our observation (Ogasawara, Mochimaru, Ueda, Ban, Kabuto, & Abe, 2016). The consistency of flavor characteristic and taste-enhancing effect of odorant was observed previously. For instance, sardine, bacon, and soy sauce aroma, could enhance the saltiness of low content NaCl solution (Lawrence et al., 2009; Nasri et al., 2011; Lee et al., 2015). On the contrary strawberry aroma associated with sweetness could not enhance the saltiness of NaCl solution (Lawrence et al., 2009; Djordjevic, Zatorre, & Jones-Gotman, 2004). The saltiness-enhancing abilities of soy sauce aroma have been confirmed in NaCl solution and foods (Kremer et al., 2009; Lee et al., 2015). However, it was still unknown which odorant played a role in the saltiness enhancement. Only one study mentioned the potential saltiness-enhancing ability of single aroma compounds (Batenburg & van der Velden, 2011). Batenburg et al. (2011) found that the saltiness of chicken bouillon could be enhanced by the odorants 4,5-dimethyl-3-hydroxy-2(5H)-furanone (sotolon), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon), furfuryl thiol and 2-methyl-3-tetrahydrofuranthiol, which were the aroma-active compounds in chicken bouillon (Feng, Cai, Fu, Zheng, Xiao, & Zhao, 2018).

Umami taste is a major characteristic of soy sauce, and the effects of odorants on umami taste were also analyzed. Figure 1b showed that OIUE was highly significant for 3-
(methylthio)propanal, dimethyl trisulfide, 3-(methylthio)-1-propanol, 1-octen-3-ol, maltol and HDMF ($p < 0.05$). It has to be reported that 3-(methylthio)propanal (also called methional) was a positive allosteric modulator of human umami taste receptor (T1R1/T1R3) (Toda, Nakagita, et al., 2018), that responded to its natural ligands, i.e. amino acids and 5'-ribonucleotides (Nelson, Nakagita, et al., 2002; Li, Staszewski, Xu, Durick, Zoller, & Adler, 2002). Therefore, the mechanism of taste enhancement observed here for this compound might be dual: odor–taste interaction and allosteric modulation of taste receptor, such as T1R1/T1R3. The umami-enhancing ability of dimethyl trisulfide was also observed by Inoue et al. (2016), who reported that dimethyl trisulfide could enhance umami aftertaste of soy miso (Japanese soybean paste). Mushroom soup or mushroom sauce were often considered to be "tasty" or "umami". This study also showed that 1-octen-3-ol with "mushroom" notes could enhance umami perception in the MSG solution. The related terms of umami were savory, meaty, broth like or salty (Ninomiya, 2002). So, as expected, salty-associated odorants enhanced umami taste perception in this study. More surprisingly, 2 sweet-associated odorants, HDMF and maltol, could also enhance the umami taste perception. This phenomenon showed that there was a potential link between umami and sweetness, possibly indicating the incongruence between odor and taste. Because the umami taste was described as "delicious" (McCabe & Rolls, 2007), and there was a strong relationship between sweetness and positive hedonic values, such as "happy", "nice", "good" (Chiva, 1987), a hypothesis would be that the sweet-associated odorants, especially caramel-like sweet odor, may increase overall pleasantness, which, in turn, may increase umami
taste.

HEMF (associated to umami) and 2,5-dimethyl-pyrazine (associated to salty taste) could enhance the umami taste of 0.3% MSG solution but did not reach the statistical significance threshold ($p > 0.05$) (Figure 1b). Some conflicting results of OIUE sensory evaluation of HEMF were found when checking the raw score data given by panelists. Around 60% of panelists confirmed the umami-enhancing ability of HEMF in 0.3% MSG solution, while the remaining 40% of panelists could not detect the higher umami tastes, even reported the umami-reducing ability of HEMF, because the latter group of panelists thought the “sweetness”, “mouthfulness” and “richness” of MSG solution, not umami taste, were enhanced by HEMF.

3.3. Saltiness-enhancing effect of 3-(methylthio)propanal as a function of NaCl concentration

The odorant with strongest saltiness-enhancing ability, 3-(methylthio)propanal was added to the solution at different NaCl concentrations, to explore if the saltiness-enhancing effect depended on salt level. The OISE of 3-(methylthio)propanal in NaCl solution at different concentrations is shown in Figure 2. OISE was significant ($p < 0.05$) at every concentration including no salt added. The highest OISE of 3-(methylthio)propanal was obtained in 0.3% salt solution; the enhancement was lower at the highest salt concentration of 0.8%. This trend was consistent with a previous study (Nasri et al., 2011), in which the saltiness of a low- or medium-salt-content solution could be enhanced significantly when panelists perceived the congruent salty aroma simultaneously, and OISE was no more significant with high-salt-content
solution. This effect has to be considered when using OISE as a strategy to reduce the salt concentration in food.

4. Conclusions

A total of 30 taste-associated compounds were identified in soy sauce by using the GC/O-AT method, which provided “taste” attributes to an odorant. The abilities of 9 selected odorants to enhance salty and umami taste were tested in NaCl or MSG solution. The results showed that 3-(methylthio)propanal, 1-octen-3-ol, 3-(methylthio)-1-propanol and 2,5-dimethylpyrazine could significantly enhance salty taste ($p < 0.05$), while 3-(methylthio)propanal, dimethyl trisulfide, 3-(methylthio)-1-propanol, 1-octen-3-ol, maltol and HDMF could significantly enhance umami taste ($p < 0.05$). The enhancement of salty taste by the cooked-potato-smelling 3-(methylthio)propanal depended on salt concentration and was the highest at the medium 0.3% salt concentration. Overall, this study may provide the food industry with a method to reduce salt in food and formulate healthier products by using soy sauce aroma. Moreover, this study verified a potential influence of soy sauce aroma on umami taste, which is quite an important quality for soy sauce.

On the basis of the present findings, further work on the effect of the soy sauce aroma recombination model on salty and umami taste, as well as the sensory tests of these taste-associated volatiles in complex food matrix, are in progress, to elucidate the effects of odor–taste interactions in soy sauce and provide valuable information for salt reduction in the food industry.
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FIGURE CAPTIONS

**Fig. 1.** Mean of the odor-induced saltiness enhancement (OISE) (a) and odor-induced umami enhancement (OIUE) (b) for the samples with an aroma compound. Error bars represent the standard error of the mean. Stars indicate significant OISE or OIUE different from 0: ***$p < 0.001$; **$p < 0.05$; *$p < 0.1$. Orange dots represent the odor intensity of each solution.

**Fig. 2.** Mean of the odor-induced saltiness enhancement (OISE) for different NaCl concentrations (0%, 0.3%, 0.8%) adding 3-(methylthio)propanal (3MPal). Error bars represent the standard error of the mean. Stars indicate significant OISE different from 0: ***$p < 0.001$; **$p < 0.05$; *$p < 0.1$. 
Table 1. Odorants identified in Chinese soy sauce by GC–MS/O and GC/O-AT.

<table>
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<tr>
<th>No</th>
<th>RI</th>
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<th>Intensity</th>
<th>Taste description</th>
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<td></td>
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<td>3-methylbutanoic acid</td>
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<td>2.8</td>
<td>sour (87%) salty (13%)</td>
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<tr>
<td>3</td>
<td>874</td>
<td>2-methylbutanoic acid</td>
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<td>2.8</td>
<td>sour (60%) sweet (20%) bitter (20%)</td>
<td>A</td>
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<tr>
<td>4</td>
<td>1319</td>
<td>4-vinylguaiacol</td>
<td>spicy, burnt</td>
<td>2.2</td>
<td>sour (34%) bitter (22%) umami (22%) salty (11%) sweet (11%)</td>
<td>A</td>
</tr>
<tr>
<td>Odor descriptors associated with sweetness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>773</td>
<td>ethyl isobutyrate</td>
<td>fruity</td>
<td>1.8</td>
<td>sweet (100%)</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>814</td>
<td>ethyl butanoate</td>
<td>fruity</td>
<td>2.3</td>
<td>sweet (83%) bitter (17%)</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>861</td>
<td>3-hexen-2-one</td>
<td>grass, geranium</td>
<td>2.3</td>
<td>sweet (72%) bitter (14%) salty (14%)</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>926</td>
<td>2-acetyl-1-pyrroline</td>
<td>cooked rice, popcorn</td>
<td>3.0</td>
<td>sweet (67%) salty (11%) umami (22%)</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>1034</td>
<td>unknown (81,99)</td>
<td>popcorn</td>
<td>2.1</td>
<td>sweet (78%) salty (22%)</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>1053</td>
<td>phenylacetaldehyde</td>
<td>floral, honey-like</td>
<td>2.0</td>
<td>sweet (100%)</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>1057</td>
<td>unknown (43,55,114)</td>
<td>cooked rice</td>
<td>2.7</td>
<td>sweet (80%) salty (20%)</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>1074</td>
<td>2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF)</td>
<td>caramel-like</td>
<td>2.0</td>
<td>sweet (86%) salty (14%)</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>1123</td>
<td>3-hydroxy-2-methyl-4-pyone (maltol) /phenylethanol</td>
<td>caramel, sweet</td>
<td>2.3</td>
<td>sweet (67%) umami (33%)</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>1133</td>
<td>isophorone</td>
<td>fruity</td>
<td>2.6</td>
<td>sweet (56%) sour (22%) salty (11%) umami (11%)</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>1158</td>
<td>2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)</td>
<td>caramel-like</td>
<td>2.4</td>
<td>sweet (75%) salty (25%)</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>1200</td>
<td>3-methoxy-2-methyl-4H-pyran-4-one</td>
<td>caramel-like</td>
<td>2.0</td>
<td>sweet (75%) bitter (25%)</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>1258</td>
<td>benzeneacetic acid</td>
<td>floral, honey-like</td>
<td>3.0</td>
<td>sweet (80%) bitter (20%)</td>
<td>B</td>
</tr>
<tr>
<td>No.</td>
<td>Mass</td>
<td>Odorant</td>
<td>Odor descriptors</td>
<td>Detection frequency (%)</td>
<td>Method of identification</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>------------------------------</td>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1271</td>
<td>tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one</td>
<td>caramel-like</td>
<td>2.0 sweet (100%)</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1285</td>
<td>4-ethyl-2-methoxyphenol (4-ethylguaiacol)</td>
<td>smoky, bacon</td>
<td>2.0 sweet (100%)</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

Odor descriptors associated with bitterness

<table>
<thead>
<tr>
<th>No.</th>
<th>Mass</th>
<th>Odorant</th>
<th>Odor descriptors</th>
<th>Detection frequency (%)</th>
<th>Method of identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>881</td>
<td>unknown (91, 98, 106)</td>
<td>medicine-like</td>
<td>3.0 bitter (75%) sour (25%)</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>1086</td>
<td>3-ethyl-2,5-dimethylpyrazine</td>
<td>nutty, burnt</td>
<td>2.8 bitter (60%) sour (20%) sweet (20%)</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>1097</td>
<td>2-methoxyphenol (guaiacol)</td>
<td>smoky, burnt</td>
<td>2.0 bitter (67%) sweet (33%)</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>1176</td>
<td>4-ethylphenol</td>
<td>phenolic</td>
<td>2.4 bitter (57%) umami (29%) sweet (14%)</td>
<td>A</td>
</tr>
</tbody>
</table>

Odor descriptors associated with saltiness

<table>
<thead>
<tr>
<th>No.</th>
<th>Mass</th>
<th>Odorant</th>
<th>Odor descriptors</th>
<th>Detection frequency (%)</th>
<th>Method of identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>897</td>
<td>unknown</td>
<td>onion</td>
<td>2.3 salty (60%) sweet (40%)</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>916</td>
<td>3-(methylthio)propanal</td>
<td>cooked potato</td>
<td>3.3 salty (78%) sweet (11%) umami (11%)</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>920</td>
<td>2,5-dimethylpyrazine</td>
<td>roasted nuts</td>
<td>2.9 salty (57%) sweet (29%) bitter (14%)</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>969</td>
<td>dimethyl trisulfide</td>
<td>cooked onion</td>
<td>2.0 salty (44%) umami (33%) sour (22%)</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>988</td>
<td>3-(methylthio)-1-propanol</td>
<td>cooked potato</td>
<td>2.4 salty (100%)</td>
<td>A</td>
</tr>
</tbody>
</table>

Odor descriptors associated with umami

<table>
<thead>
<tr>
<th>No.</th>
<th>Mass</th>
<th>Odorant</th>
<th>Odor descriptors</th>
<th>Detection frequency (%)</th>
<th>Method of identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>988</td>
<td>1-octen-3-ol</td>
<td>mushroom</td>
<td>1.8 umami (71%) bitter (29%)</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>1151</td>
<td>5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone (HEMF)</td>
<td>caramel-like</td>
<td>2.3 umami (67%) sweet (33%)</td>
<td>A</td>
</tr>
</tbody>
</table>

*a Retention index

*b Detection frequency (%): The denominator is the number of panelists who perceived the odorant, and the numerator is the number of panelists who perceived the odorant associated with a certain taste.

*c Method of identification: (A) each component was identified by comparing its RI, mass spectrum, and odor quality to those of the reference compounds; (B) tentative identification was based on comparisons of mass spectra with those of compounds from the standard NIST 08 library and the RI sourced from NIST Standard Reference Database.
<table>
<thead>
<tr>
<th>No.</th>
<th>Abbreviation</th>
<th>Odorant</th>
<th>Concentration (μg/L)</th>
<th>Associated taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PA</td>
<td>phenylacetaldehyde</td>
<td>1720</td>
<td>Sweet</td>
</tr>
<tr>
<td>2</td>
<td>Maltol</td>
<td>3-hydroxy-2-methyl-4-pyrene</td>
<td>1296</td>
<td>Sweet</td>
</tr>
<tr>
<td>3</td>
<td>HDMF</td>
<td>2,5-dimethyl-4-hydroxy-3(2H) - furanone</td>
<td>1775</td>
<td>Sweet</td>
</tr>
<tr>
<td>4</td>
<td>3MPal</td>
<td>3-(methylthio)propanal</td>
<td>855</td>
<td>Salty</td>
</tr>
<tr>
<td>5</td>
<td>1o3ol</td>
<td>1-octen-3-ol</td>
<td>739</td>
<td>Umami</td>
</tr>
<tr>
<td>6</td>
<td>3MPaol</td>
<td>3-(methylthio)-1-propanol</td>
<td>1526</td>
<td>Salty</td>
</tr>
<tr>
<td>7</td>
<td>DMTS</td>
<td>dimethyl trisulfide</td>
<td>339</td>
<td>Salty</td>
</tr>
<tr>
<td>8</td>
<td>DMPy</td>
<td>2,5-dimethylpyrazine</td>
<td>1708</td>
<td>Salty</td>
</tr>
<tr>
<td>9</td>
<td>HEMF</td>
<td>5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone</td>
<td>3220</td>
<td>Umami</td>
</tr>
</tbody>
</table>

Table 2. Concentration of selected odorants added in NaCl solution and MSG solution.
Fig. 1.
Fig. 2.
CRediT author statement

Ting Zhou: Experiment manipulation, Data analysis, Writing - original

Yunzi Feng: Experiment design, Data analysis, Writing - review & editing, Project administration, Supervision.

Thierry Thomas-Danguin: Methodology, Revise, Technical support.

Mouming Zhao: Project administration, Supervision.
Highlights

Thirty taste-associated odorants were identified from soy sauce by GC/O-AT.
Four saltiness-enhancing and six umami-enhancing odorants were detected.
3-(Methylthio)propanal was the most effective odorant in enhancing salty and umami.
The umami-associated odorant 1-octen-3-ol could enhance saltiness significantly.