Research Square

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Mixed Volvariella volvacea and Lentinula edodes extracts improve antioxidant activity and sensory quality of soy sauce Yangzhou Institute for Food and Drug Control

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Abstract

Edible mushroom extracts are promising functional food ingredients, but their incorporation into fermented condiments must preserve sensory quality. This study evaluated blends of *Volvariella volvacea* and *Lentinula edodes* extracts in soy sauce. Practical extraction conditions were screened by single-factor experiments, and concentrates were blended at V:L volume ratios of 1:3, 2:2, and 3:1 before addition to soy sauce at 2% (v/v). The 3:1 formulation (M3) had the highest measured total saccharide content (47.16 g/100 mL) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (29.18%). Total phenolic content was numerically highest in M3 (472.85 mg/L) but did not differ significantly among formulations. M3 DPPH activity was 57.8% higher than that of the control. Compared with the control, M3 showed higher electronic-tongue responses for umami and saltiness and lower responses for sourness and astringency. Sensory evaluation likewise gave M3 the highest cumulative umami (41) and saltiness (55) scores and the lowest sourness score (7), while retaining a clear soy sauce-like aroma and a moderate mushroom note. Principal component analysis separated M2 and M3 from the control, and exploratory kernel alignment showed the strongest formulation-level agreement between the electronic-tongue and selected sensory patterns. These findings support a *V. volvacea*-rich blend as a mushroom-derived functional ingredient for soy sauce, although antioxidant evidence remains limited to an *in vitro* chemical assay.

Introduction

Soy sauce is a fermented condiment made mainly from soybeans, wheat, and brine. During fermentation, these raw materials are converted into free amino acids, peptides, organic acids, and volatile compounds that determine taste and aroma [1, 2]. More than 170 volatile compounds have been reported, including aldehydes, alcohols, esters, ketones, and sulfur-containing compounds [3]. Soy sauce therefore provides a complex plant-based food matrix in which a functional ingredient must improve composition without masking the characteristic fermented flavor.

Edible mushrooms are valued as both foods and sources of functional ingredients. They contain amino acids, 5′-nucleotides, peptides, polysaccharides, phenolic compounds, and aroma-active volatiles [4, 5]. These components contribute to umami, aroma, mouthfeel, and *in vitro* antioxidant activity. Recent work has evaluated edible mushrooms as natural umami sources using free amino acids, 5′-nucleotides, equivalent umami concentration, and sensory analysis [6]. Extracts of *Volvariella volvacea* and *Lentinula edodes* may therefore enrich fermented condiments while also modifying their sensory profile.

The use of mushroom extracts in soy sauce requires a balance between functional composition and sensory quality. Higher phenolic levels may improve chemical antioxidant activity but can also increase bitterness or astringency [7, 8]. Strong mushroom aroma may also mask the fermented identity of soy sauce. Previous studies have mainly examined individual mushroom species or single processing treatments [9]. The effects of mixed *V. volvacea* and *L. edodes* extracts at a fixed, low addition level have

received less attention, particularly with respect to the joint changes in antioxidant activity, instrumental flavor responses, and sensory quality.

This study therefore examined mixed *V. volvacea* and *L. edodes* extracts as functional ingredients for soy sauce. Single-factor experiments were used to screen practical extraction conditions based on total saccharide content (TSC), total phenolic content (TPC), and DPPH radical-scavenging activity. Three concentrate ratios were then compared at a fixed total addition level of 2% (v/v). Electronic-nose, electronic-tongue, sensory, and multivariate analyses were used to evaluate whether the mixtures improved functional and sensory quality while retaining the characteristic soy sauce profile.

Materials and methods

This section is provided as Supplementary Material 1 (S1).

Results and discussion

Optimization of extraction conditions

Extraction conditions were first optimized to obtain mushroom extracts with high bioactive content and suitable process efficiency. For application, the extract should provide enough TSC, TPC, and antioxidant activity without using excessive solvent (Fig. 1). The solid-to-liquid ratio was therefore evaluated using both concentration-based values and dry-weight yield. This approach reduces the effect of simple dilution and gives a more practical basis for selecting process conditions.

Effect of solid-to-liquid ratio The solid-to-liquid ratio strongly affected extraction performance (Fig. 1a-c). As shown, the bars show the concentration of TSC, TPC, and DPPH radical scavenging activity in the extracts, whereas the lines show the corresponding values normalized to mushroom dry weight. The extract concentrations decreased as the ratio changed from 1:10 to 1:50 (w/w). For *V. volvacea* extract (VVE), TSC decreased from 26.28 ± 0.49 to 8.15 ± 0.70 g/L, TPC decreased from 1.08 ± 0.01 to 0.21 ± 0.01 mg GAE/mL, and DPPH radical scavenging activity decreased from 39.32 ± 0.80% to 16.04 ± 0.78%. For *L. edodes* extract (LEE), TSC decreased from 16.01 ± 0.57 to 3.90 ± 0.38 g/L, TPC decreased from 0.61 ± 0.03 to 0.13 ± 0.01 mg GAE/mL, and DPPH radical scavenging activity decreased from 62.91 ± 1.01% to 24.93 ± 0.51%. Thus, 1:10 gave the most concentrated extracts, which is important because the concentrates were later added to soy sauce at a low level.

The dry-weight-normalized data gave a complementary view of extraction efficiency. For TSC, the dry-weight values increased at higher solvent volumes and reached about 407.38 mg/g DW for VVE and 195.17 mg/g DW for LEE at 1:50. This indicates that a larger solvent volume could extract more soluble sugars from the raw material, even though the final extract was more dilute. For TPC, the highest dry-weight-normalized values were observed at 1:30, reaching 12.28 ± 0.72 mg GAE/g DW for VVE and 10.81 ± 0.02 mg GAE/g DW for LEE. In contrast, the VCE-equivalent antioxidant values were highest at 1:10, with 11.67 ± 0.10 mg VCE/g DW for VVE and 14.33 ± 0.13 mg VCE/g DW for LEE, and then decreased as

solvent volume increased. These results show a trade-off between absolute extraction yield and extract strength. Because this study aimed to prepare a concentrated flavoring ingredient for soy sauce, 1:10 was selected as the most practical ratio.

From a processing point of view, a higher solvent ratio would also increase solvent use and later concentration cost. Therefore, a solid-to-liquid ratio of 1:10 was selected for the following experiments.

Effect of extraction temperature Temperature can affect compound release, diffusion, and the stability of heat-sensitive compounds. As shown in Fig. 1d-f, extraction temperatures of 40, 60, and 80°C were compared using TSC, TPC, and DPPH radical scavenging activity as indices. Because the solid-to-liquid ratio was fixed at 1:10, the dry-weight-normalized values followed the same general pattern as the extract concentrations.

For LEE, TSC changed only slightly with temperature, ranging from 16.62 ± 1.13 to 17.45 ± 0.79 g/L. However, TPC decreased clearly as temperature increased. It declined from 0.53 ± 0.03 mg GAE/mL at 40°C to 0.36 ± 0.01 mg GAE/mL at 80°C. DPPH radical scavenging activity also decreased from 35.85 ± 5.64% at 40°C to 17.38 ± 2.06% at 80°C. These data indicate that higher temperature did not improve the extraction of antioxidant-related compounds from LEE.

For VVE, TSC and TPC reached their numerical peaks at 60°C. TSC increased from 29.24 ± 1.32 g/L at 40°C to 31.36 ± 2.78 g/L at 60°C, and then decreased to 26.61 ± 0.33 g/L at 80°C. TPC followed a similar trend, increasing from 0.78 ± 0.05 to 0.82 ± 0.04 mg GAE/mL between 40 and 60°C, and then decreasing to 0.65 ± 0.02 mg GAE/mL at 80°C. DPPH radical scavenging activity was also higher at 60°C (40.97 ± 3.46%) than at 40°C (35.73 ± 3.66%), but both temperatures shared the same significance group in Fig. 2b. Therefore, the small gain at 60°C did not justify the higher heating input. Considering both activity retention and energy use, 40°C was selected as the extraction temperature.

Effect of ultrasonication duration Ultrasonic-assisted extraction can improve mass transfer through cavitation. However, long treatment may also increase local heating and damage some target compounds [10]. Therefore, ultrasonication times of 30, 60, and 90 min were compared using the same three indices (Fig. 1g-i).

For LEE, extending ultrasonication from 30 to 90 min caused little change in the three indices. TSC was 12.85 ± 1.00 g/L at 30 min, 13.74 ± 0.45 g/L at 60 min, and 13.28 ± 0.96 g/L at 90 min. TPC remained close to 0.45 mg GAE/mL, and DPPH radical scavenging activity stayed within a narrow range from 30.86% to 31.66%. These results show that most extractable compounds in LEE were released within the first 30 min.

For VVE, 30 min also gave high extraction values. TSC was 33.23 ± 0.60 g/L at 30 min and decreased to 31.43 ± 1.06 g/L at 60 min and 28.21 ± 0.58 g/L at 90 min. TPC and DPPH radical scavenging activity changed only slightly across the tested times: TPC ranged from 0.76 ± 0.04 to 0.80 ± 0.01 mg GAE/mL, and DPPH radical scavenging activity ranged from 41.49 ± 3.64% to 44.05 ± 1.96%. Because longer

ultrasonication did not provide a clear improvement, 30 min was selected as the extraction time for both mushroom extracts.

Bioactive profile of mushroom-flavored soy sauce

LEE and VVE were prepared under the selected extraction conditions and concentrated before use. The two concentrates were then mixed at different V:L ratios (1:3, 2:2, 3:1) and added to soy sauce at a fixed total level of 2% (v/v). This design allowed the effect of the V:L ratio on bioactive and sensory quality to be compared.

TSC differed significantly among formulations (**Fig. 2a**). It increased from 8.33 ± 0.93 g/100 mL in the control to 37.31 ± 1.74 g/100 mL in M1, 37.58 ± 4.82 g/100 mL in M2, and 47.16 ± 1.46 g/100 mL in M3, with M3 showing the highest value. TPC was numerically higher in the mushroom-enriched samples, increasing from 420.98 ± 50.71 mg/L in the control to 458.71 ± 19.93, 465.14 ± 27.80, and 472.85 ± 27.18 mg/L in M1, M2, and M3, respectively; however, these differences were not significant ($p > 0.05$; **Fig. 2b**). DPPH activity was 18.49 ± 4.02% in the control, 17.55 ± 2.53% in M1, 19.61 ± 2.85% in M2, and 29.18 ± 7.59% in M3. Only M3 differed significantly from the other formulations ($p < 0.05$; **Fig. 2c**).

These data show that the antioxidant response was not caused by mushroom addition alone, but depended on the V:L ratio. M1 did not exceed the control in DPPH radical scavenging activity, the value of M2 was merely 6.1% higher than that of the control, whereas M3 increased this value by about 57.8% relative to the control. This result supports the use of a higher VVE proportion when antioxidant capacity is a target. However, phenolic compounds can also increase bitterness and astringency when present at high levels [11]. For a gastronomic condiment, this trade-off is important. A formulation should not only increase bioactive compounds but also keep a pleasant taste and aroma.

LEE may help improve this balance because *L. edodes* contains taste-active nucleotides and aroma compounds[12] These compounds can support umami and add mushroom-like aroma. In the soy sauce matrix, the role of LEE is therefore not only to add bioactive compounds but also to help shape the flavor profile.

Taken together, the M3 ratio provided a useful balance. It kept high TSC, TPC, and DPPH radical scavenging activity among the mixed formulations, while also retaining enough LEE to support umami and aroma depth. This balance is more relevant to soy sauce use than maximizing a single chemical index.

Flavor profile analysis

Electronic nose analysis of aroma profile The electronic nose was used to compare the overall volatile response patterns of the control and the three mixed-formulation soy sauce samples. PEN3 sensors are semi-selective metal oxide sensors. Therefore, their signals were interpreted as sensor response classes rather than as direct identification of individual volatile compounds.

As shown in **Fig. 3a**, the control soy sauce showed the strongest responses on W1W and W2W, with mean values of 9.52 ± 2.08 and 5.61 ± 0.81, respectively. W1W is mainly associated with sulfur compounds, whereas W2W responds to aromatic hydrocarbons and organosulfur compounds. After the addition of mixed mushroom concentrates, W1W decreased to 7.46 ± 0.71 in M1, 7.62 ± 0.09 in M2, and 7.60 ± 0.51 in M3. W2W also decreased to 4.51 ± 0.31, 4.77 ± 0.05, and 4.75 ± 0.22, respectively. These data indicated that the mixed concentrates moderated the sulfur-related sensor responses of soy sauce rather than simply increasing the fermented odor intensity.

The main non-sulfur changes were observed in W1C, W3C, W5C, W5S, and W2S. W1C, which is mainly sensitive to aromatic organic compounds, increased from 0.37 ± 0.01 in the control to 0.40 ± 0.02 in M1, 0.39 ± 0.01 in M2, and 0.40 ± 0.01 in M3. W3C, which is associated with ammonia and aromatic molecules, increased slightly from 0.73 ± 0.02 to 0.76 ± 0.02, 0.74 ± 0.02, and 0.75 ± 0.01, respectively. W5C, which responds to short-chain alkanes/alkenes and aromatic compounds, changed only slightly (0.85 ± 0.02 in the control and approximately 0.86 in the mixed groups). By contrast, W5S, a broad-range sensor mainly sensitive to nitrogen oxides, decreased from 2.63 ± 0.23 in the control to approximately 2.44 in the mixed groups.

The W2S response, which is commonly associated with alcohol-related compounds and partly aromatic broad-range volatiles, also decreased from 2.62 ± 0.09 in the control to 2.45 ± 0.13 in M1, 2.47 ± 0.08 in M2, and 2.39 ± 0.01 in M3. Other sensors showed smaller changes: W6S remained near 1.13, W1S remained close to 1.00, and W3S remained near 1.13. Together, these results showed that mixed mushroom concentrates reshaped the aroma response pattern mainly by reducing sulfur-related and alcohol-related broad-range responses while slightly increasing aromatic-response signals. This pattern was consistent with a more balanced mushroom-flavored soy sauce aroma.

Electronic tongue analysis of taste attributes The electronic tongue was used to measure eight taste-related responses: sourness, bitterness, astringency, bitter aftertaste, astringent aftertaste, umami, richness, and saltiness. These instrumental responses were used to compare how the three V:L ratios changed the taste profile of soy sauce relative to the control.

As shown in **Fig. 3b**, the control soy sauce showed a high sourness response (35.97), moderate umami (62.21), and moderate saltiness (59.09). M1 was close to the control for most taste attributes: sourness was 36.86, umami was 62.18, and saltiness was 59.20. This indicates that the 1:3 V:L ratio had only a limited effect on the electronic tongue taste pattern.

M2 and M3 produced a clearer taste shift. Sourness decreased sharply from 35.97 in the control to 10.77 in M2 and 10.30 in M3. At the same time, umami increased from 62.21 to 69.59 and 69.12, respectively, while saltiness increased from 59.09 to 65.91 and 64.92. Astringency also decreased from 57.51 in the control to 51.15 in M2 and 50.27 in M3. These data show that higher VVE proportions improved the main positive taste responses and reduced sourness and astringency.

Bitterness required a more cautious interpretation. The electronic tongue bitterness response increased from 67.75 in the control to 70.45 in M2 and 71.03 in M3, whereas bitter aftertaste changed only slightly (59.34 in the control, 58.92 in M2, and 58.56 in M3). Richness also remained stable, ranging from 59.41 to 59.76 across the control and mixed formulations. Therefore, the main instrumental benefit of M3 was not a lower bitterness signal, but a better balance between high umami/saltiness and low sourness/astringency. This supports the use of M3 as the preferred taste formulation.

Sensory evaluation of flavored soy sauce Sensory evaluation was used to link instrumental responses with human perception. Thirty-four trained assessors scored nine attributes on a 0-2 scale for the control and the three mixed-formulation samples, so the cumulative score for each attribute could range from 0 to 68 (**Fig. 3c**).

The sensory data generally supported the electronic tongue results, especially for umami, saltiness, and sourness. M3 had the highest umami scores among the control and mixed formulations, reaching 41. Its saltiness scores were also the highest, reaching 55, compared with about 52 for the control. Sourness was lower in M3 (about 7) than in the control (about 12) and M1 (about 12). These sensory scores agree with the electronic tongue results, in which M3 showed higher umami and saltiness and much lower sourness.

The sensory bitterness and astringency scores showed an important difference from the instrumental bitterness response. Although the electronic tongue bitterness response was higher in M2 and M3, the panel still gave relatively low bitterness scores to the mixed formulations. M1 showed the lowest bitterness score (about 5), whereas M2 and M3 were about 9. Astringency was moderate in the mixed groups, with M3 scoring about 14, close to or below the control. This suggests that the human-perceived bitter/astringent impact was partly masked by stronger umami and saltiness [13].

The aroma-related sensory scores further supported M3. A useful mushroom-flavored soy sauce should keep a clear soy sauce-like aroma while adding only a moderate mushroom note. M3 had a soy sauce-like aroma score of 40, close to M2 and slightly higher than the control. Its mushroom-like score was low (about 11), and its off-flavor score was also low to moderate (about 9). Taken together, M3 increased umami and saltiness, reduced sourness, and kept the aroma profile balanced. This makes it the most suitable mixed formulation for a culinary soy sauce, where overall balance is more important than the highest value for any single attribute.

Multivariate differentiation of instrumental flavor profiles

Principal component analysis (PCA) PCA was used to visualize the overall differences in the instrumental flavor profiles of the soy sauce samples. The analysis was based on standardized electronic-nose and electronic-tongue responses. As shown in **Fig. 4a**, PC1 and PC2 explained 45.6% and 26.3% of the total variance, respectively. Their cumulative contribution reached 71.9%, indicating that the first two components captured the main variation in the instrumental dataset.

The score plot showed a clear distribution according to the mushroom extract ratio. The control replicates were located on the positive side of PC1 and were distributed across the first and fourth quadrants. M1 was mainly located in the fourth quadrant, with one replicate extending towards the third quadrant. In contrast, M2 and M3 clustered in the second quadrant, showing negative PC1 scores and positive PC2 scores. This spatial shift from C to M1 and then to M2/M3 indicated that increasing the proportion of VVE progressively changed the combined aroma and taste profile. M3 formed a compact cluster and was positioned farthest from the control, suggesting the most consistent instrumental flavor shift among the mixed formulations.

The loading plot explained the variables associated with this separation (**Fig. 4b**). Positive PC1 loadings were observed for sourness, astringency, bitter aftertaste, richness, and several sulfur- or broad-range volatile sensor responses, including W1W, W2W, W2S, W3S, W5S, and W6S. These variables were oriented towards the control samples. Negative PC1 loadings were observed for umami, saltiness, instrumental bitterness, W1C, W1S, W3C, and W5C and were oriented towards the mixed formulations. PC2 further separated M1 from M2 and M3. Its positive side was associated with umami, saltiness, instrumental bitterness, W6S, W5S, W2W, W1W, W3S, and W2S, whereas its negative side was associated with W1C, W1S, W3C, W5C, sourness, astringency, richness, and bitter aftertaste. PCA therefore showed that formulation differences were mainly related to the balance between umami and saltiness, aroma-sensor responses, and sour or astringent taste responses. Together with the sensory results, this pattern supported M3 as the most balanced formulation.

Exploratory agreement between instrumental and sensory patterns Centered linear kernel alignment (CKA) was used to quantify whether the similarity pattern among the four formulations in each instrumental block agreed with sensory umami and soy sauce-like aroma (**Fig. 5a**). The analysis used one mean instrumental profile and one sensory profile for each formulation, thereby keeping the instrumental and sensory data at the same experimental level. CKA ranges from 0 to 1, with higher values indicating stronger agreement between two multivariate configurations.

The electronic-tongue block showed the strongest alignment with the sensory data. Its CKA coefficient was 0.822 for soy sauce-like aroma (exact $p = 0.042$), 0.805 for sensory umami ($p = 0.083$), and 0.861 for the joint sensory profile ($p = 0.083$). The observed electronic-tongue alignment with soy sauce-like aroma was the largest value among all 24 label permutations (**Fig. 5b**). The p values were necessarily coarse because only four formulations were available.

The electronic-nose block showed weaker agreement with the selected sensory targets, with CKA coefficients of 0.055 for soy sauce-like aroma ($p = 1.000$), 0.345 for sensory umami ($p = 0.458$), and 0.212 for the joint profile ($p = 0.667$). The combined instrumental block gave intermediate coefficients of 0.520, 0.721, and 0.656, respectively, but none reached the smallest attainable exact p value. Adding the electronic-nose variables therefore did not improve alignment beyond that obtained with the electronic tongue alone. This result does not imply that volatile compounds were unimportant; it only shows that

the electronic-nose similarity pattern did not closely follow these two aggregate sensory scores across the four tested formulations.

At the formulation level, M3 had the highest sensory umami score (41) and soy sauce-like aroma score (40). PCA also placed M3 farthest from the control in the direction associated with instrumental umami and saltiness. The kernel-alignment results indicate that the sensory progression across C, M1, M2, and M3 was more consistent with the electronic-tongue block than with the electronic-nose block. Together, these analyses support the conclusion that the flavor improvement in M3 was driven mainly by taste modulation while the characteristic soy sauce-like aroma was retained.

Implications for functional food formulation

At a fixed addition level of 2% (v/v), the 3:1 V:L blend produced the strongest combination of in vitro antioxidant activity and sensory quality. This result indicates that species ratio is important when mushroom extracts are used as functional food ingredients. Addition alone was insufficient: M1 did not improve DPPH activity over the control, whereas M3 increased DPPH activity by 57.8% and retained a clear soy sauce-like aroma.

The stronger umami and saltiness responses of M3 may be useful in the design of fermented condiments with a richer taste profile. However, the study did not measure sodium reduction, storage stability, bioaccessibility, or health effects. Further work should verify the formulation in reduced-sodium products and real food matrices and should characterize the specific saccharides, phenolics, amino acids, nucleotides, and volatile compounds responsible for the observed effects.

Conclusion

Mixed *V. volvacea* and *L. edodes* extracts were evaluated as functional ingredients for soy sauce. Single-factor screening identified a solid-to-liquid ratio of 1:10 (w/w), 40°C, and 30 min of ultrasonication as practical extraction conditions. Among the three blends, M3 (V:L = 3:1) showed the strongest overall performance. It had the highest measured TSC and DPPH radical-scavenging activity, and its DPPH activity was 57.8% higher than that of the control. M3 also showed stronger umami and saltiness responses, lower sourness, and a clear soy sauce-like aroma with a moderate mushroom note. PCA distinguished M2 and M3 from the control, while exploratory kernel alignment showed the closest formulation-level agreement between the electronic-tongue and sensory patterns. These results support a *V. volvacea*-rich blend as a mushroom-derived ingredient for functional fermented condiments. Further studies should confirm compound identity, bioaccessibility, storage stability, and performance in reduced-sodium products and real food matrices.

Abbreviations

ANOVA
analysis of variance

CKA
centered linear kernel alignment
DPPH
2,2-diphenyl-1-picrylhydrazyl
DW
dry weight
LEE
L. edodes extract
PCA
principal component analysis
SD
standard deviation
TPC
total phenolic content
TSC
total saccharide content
VCE
vitamin C equivalents
VVE
V. volvacea extract

Declarations

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Author contributions Xinyu Wu: Investigation, Formal analysis & Writing – Original Draft; Yuqi Xie: Methodology, Visualization, & Writing – Original Draft; Dingshuo Zheng: Methodology, Visualization, Validation & Writing – Review & Editing; Jiaxiu Wang: Software, Resources, & Writing - Review & Editing. Qiangshen Xu: Data curation, Visualization, Validation & Writing – Review & Editing; Shuai Wang: Project administration, Software, & Writing - Review & Editing; Shuo Wang: Funding acquisition, Conceptualization, & Writing – Original Draft.

Data availability Data will be made available on request.

Ethical approval This study was performed in compliance with the Declaration of Helsinki and the Ethical Review Measures for Life Sciences and Medical Research Involving Humans (China). Formal ethical approval was not required as the study met the ethical exemption requirements specified in

Article 32, as confirmed by the Ethics Committee of Yangzhou University. Informed consent was obtained from all participants, and no allergens were involved.

Competing interest The authors declare no competing interests.

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Figures

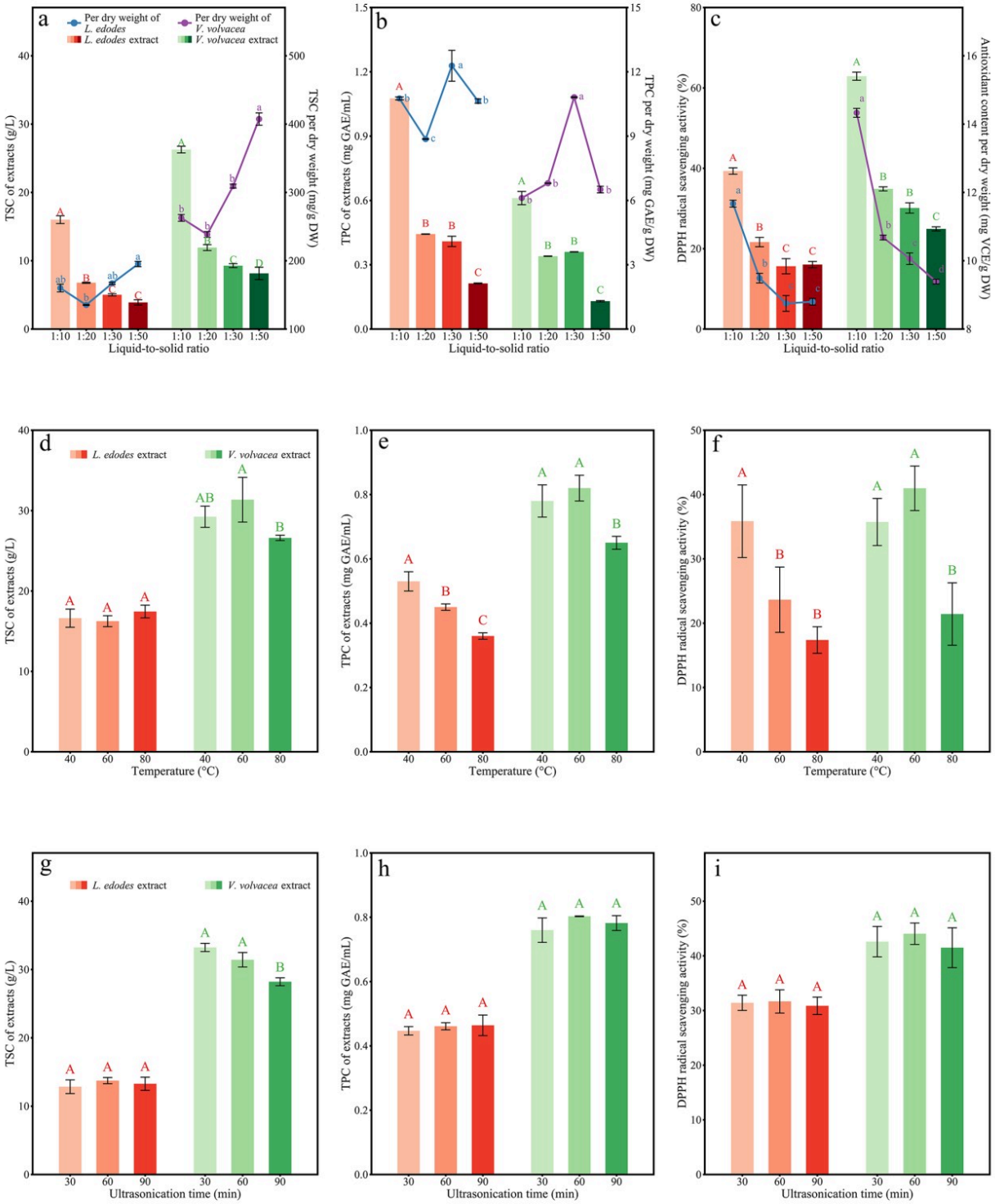


Figure 1

Effects of extraction conditions on *L. edodes*and *V. volvacea* extracts: (a-c) solid-to-liquid ratio, (d-f) extraction temperature, and (g-i) ultrasonication time. Columns show total saccharide content (a, d, g), total phenolic content (b, e, h), and DPPH radical-scavenging activity (c, f, i). In a-c, bars show extract concentrations on the left y-axis and lines show dry-weight-normalized values on the right y-axis. Values

are mean ± SD (n = 3). Different letters indicate significant differences among conditions within each mushroom species ($p < 0.05$)

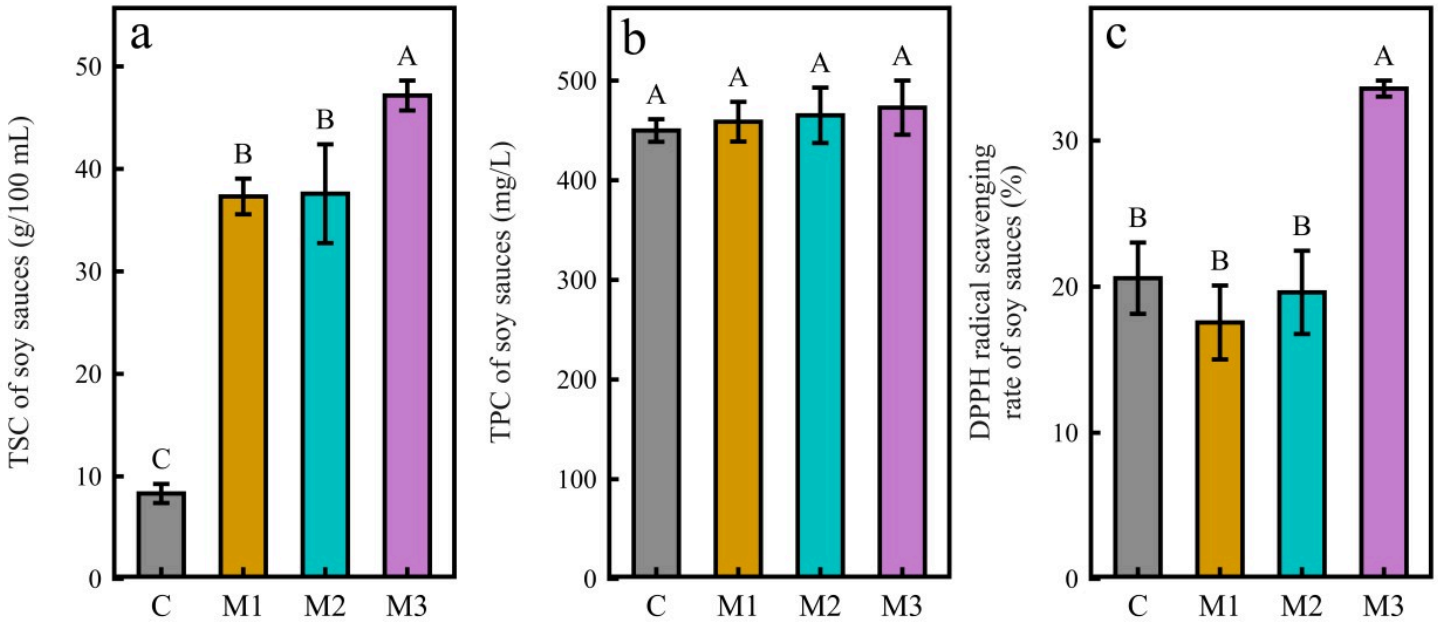


Figure 2

Total saccharide content (TSC, a), total phenolic content (TPC, b), and DPPH radical-scavenging activity (c) of the control soy sauce (C) and soy sauces containing *V. volvacea*:*L. edodes* concentrate blends at 1:3 (M1), 2:2 (M2), and 3:1 (M3). Values are mean ± SD (n = 3). Different letters indicate significant differences among formulations ($p < 0.05$)

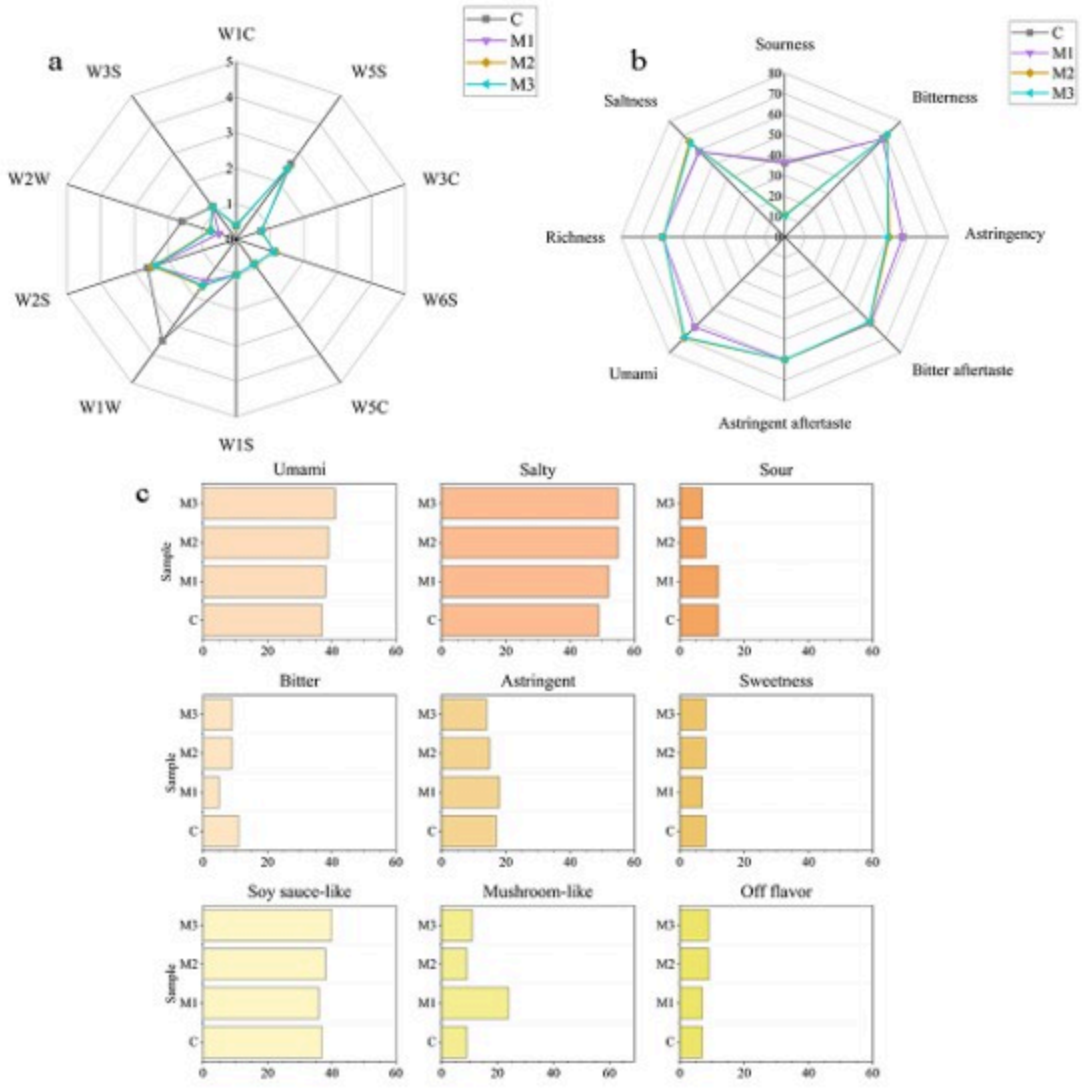


Figure 3

Instrumental and sensory profiles of the control (C) and mushroom-enriched soy sauces (M1-M3): (a) electronic-nose responses, (b) electronic-tongue responses, and (c) cumulative sensory scores from 34 assessors. M1, M2, and M3 contained *V. volvacea*:*L. edodes* concentrate blends at 1:3, 2:2, and 3:1, respectively

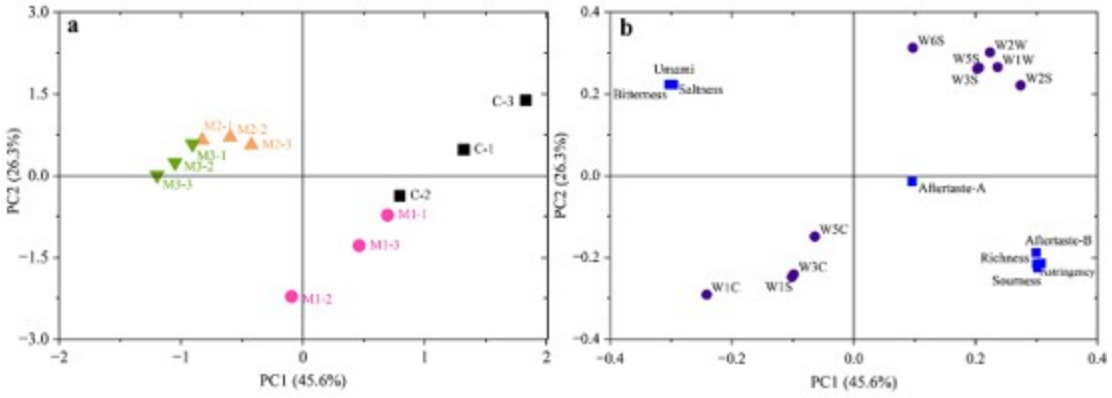


Figure 4

Principal component analysis of standardized electronic-nose and electronic-tongue responses: (a) score plot and (b) loading plot. C, control soy sauce; M1-M3, soy sauces containing *V. volvacea*:*L. edodes* concentrate blends at 1:3, 2:2, and 3:1, respectively

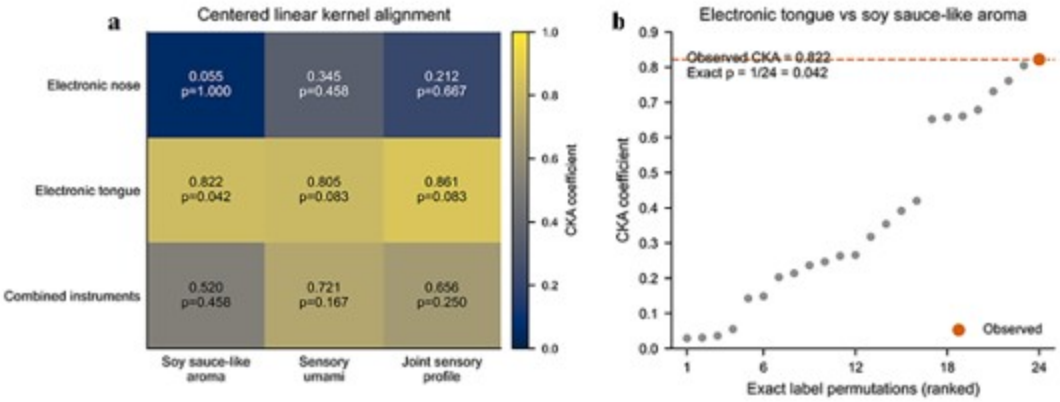


Figure 5

Multivariate analysis of instrumental flavor profiles and formulation-level sensory alignment: (a) Centered linear kernel alignment between formulation-mean instrumental blocks and sensory soy sauce-like aroma, sensory umami, and their joint profile; (b) Exact permutation distribution for the strongest observed alignment, between the electronic-tongue block and soy sauce-like aroma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx