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Characterization of the Pure Black Tea Wine Fermentation Process by Electronic Nose and Tongue-Based Techniques with Nutritional Characteristics

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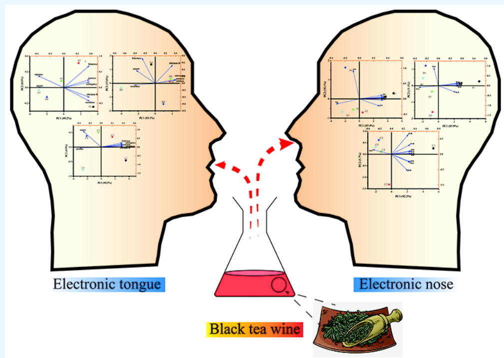
ABSTRACT: Wine is an alcoholic beverage, consisting of several compounds in various ranges of concentrations. Wine quality is usually assessed by a sensory panel of trained personnel. Electronic tongues (e-tongues) and electronic noses (e-noses) have been established in recent years to assess the quality of beverages and foods. Response surface and electronic analysis tools were used to examine the quality of black tea wine. The results indicated the optimum initial sugar level (25 °Brix), yeast addition (0.5%), and fermentation temperature (25 °C) for Golden Peony black tea wine. The black tea wine produced under these conditions with 14.0% vol alcohol has as an orange-red color, full wine and tea flavor, and mild and mellow taste. The sourness of the wine was most affected by fermentation factors—yeast addition, fermentation temperature, and initial sugar level. Alcohols, aldehydes, ketones, and alkanes contributed to most of the volatile components under the influence of yeast addition and fermentation temperature. In contrast, nitrogen oxides, aromatics, and organic sulfides contributed under the influence of the initial sugar level. This study provided a facilitated strategy for obtaining the optimum black tea wine fermentation process through electronic nose and tongue-based techniques. The analysis of wines requires new technologies able to detect various different compounds simultaneously, providing worldwide information about the sample instead of information about specific compounds.



1. INTRODUCTION

Black tea is one of the most popular beverages around the globe. The six major tea types in China are green, white, yellow, oolong, black, and dark tea.[1] Black tea is obtained through complex enzymatic oxidation and polymerization reactions between various enzymes through withering, kneading, and fermentation.[2] It is well known in the Chinese consumer market for its sweet taste, warmth, stomach warming, and easy preservation. Nevertheless, nearly 70% of market output is sold as middle- and high-grade tea and the remaining part as low-grade tea, leading to problems such as its stagnation and continuous price decline. Therefore, upgrading the utilization value of stagnant tea is an issue worth focusing on.

The organic combination of tea and wine produces a distinctive tea scent and multiple physiological functions, as well as promotes the diversification of wine.[3] According to the brewing process, tea wines can be categorized into three groups such as sparkling, prepared, and fermented. The research mainly focuses on optimizing rice[4] and composite tea wines.[5,6] However, the studies on the fermentation of tea wine using pure tea juice have only analyzed the chemical composition, but no studies on the taste of tea wine are being reported.

Wine fermentation is an extremely complex biochemical process involving yeast. The quality of wine is known to be influenced by several factors, like the initial sugar level, cultural condition, and yeast addition.[5] Therefore, these factors need to be optimized in depth. The electronic nose and tongue sensors mainly mimic the human senses of smell and taste, providing rapid detection and global information about the sample.[7,8] This technique has been widely used for quality testing in foods, such as fruits,[9] wine,[10] and tea beverages.[11] However, no research has been reported on the use of this technique for tea wine quality identification.

This study was conducted to prepare fermented tea wine from tea juice, and single-factor tests investigated the main factors affecting the quality of tea wine. The flavor of tea wine was evaluated using electronic nose and tongue-based techniques, and the variety of tastes was examined by principal

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component analysis (PCA). Finally, the tea wine flavor to better suit consumer demand and increase the added value of tea, response surface analysis was used to identify the optimum tea wine fermentation process. The results were expected to provide a new technical method for the quality control and evaluation of tea wine.

2. EXPERIMENTAL SECTION

2.1. Black Tea Wine Preparation. Black tea was provided by the main tea-producing areas of Hezhou Bapu District and Zhaoping County, China. White granulated sugar was produced by Nanning Sugar Industry Co. RW Commercial Yeast was purchased from Anchor Yeast Co. Black tea and water were extracted at 80 °C for 2 h at a material-to-liquid ratio of 1:60. The tea broth was used as the fermentation base and pasteurized for 30 min after adjusting the sugar content (solid soluble content, SSC) to 30 °Brix. When the tea broth was cooled at room temperature, 0.1% activated yeast was added and fermented at 25 °C.[12] The dry yeast is activated for 15−30 min with more than 5 times 2% sugar water during 35−38 °C.[13]

Table 1. Factors and Levels Used in the Box−Behnken Design

	factors		
	A	B	C
levels	yeast addition (%)	initial sugar (°Brix)	fermentation temperature (°C)
−1	0.1	20	20
0	0.5	25	25
1	1	30	30

2.2. Single-Factor Experiments. A preliminary investigation providing the factors affecting the alcoholic content and inclusions of fermented black tea wine was conducted using single-factor experiments, including the initial sugar level, yeast addition, and fermentation temperature. The factors chosen as the initial sugar level (15, 20, 25, 30, and 35 °Brix), yeast addition (0.1, 0.5, 1.0, 1.5, and 2.0%), and temperature of fermentation (15, 20, 25, 30, and 35 °C) played a significant role on the quality of black tea wine.

2.3. Box−Behnken Design (BBD). Based on the key factors and levels of the test determined by single-factor experiments, a three-factor, three-level Box−Behnken design

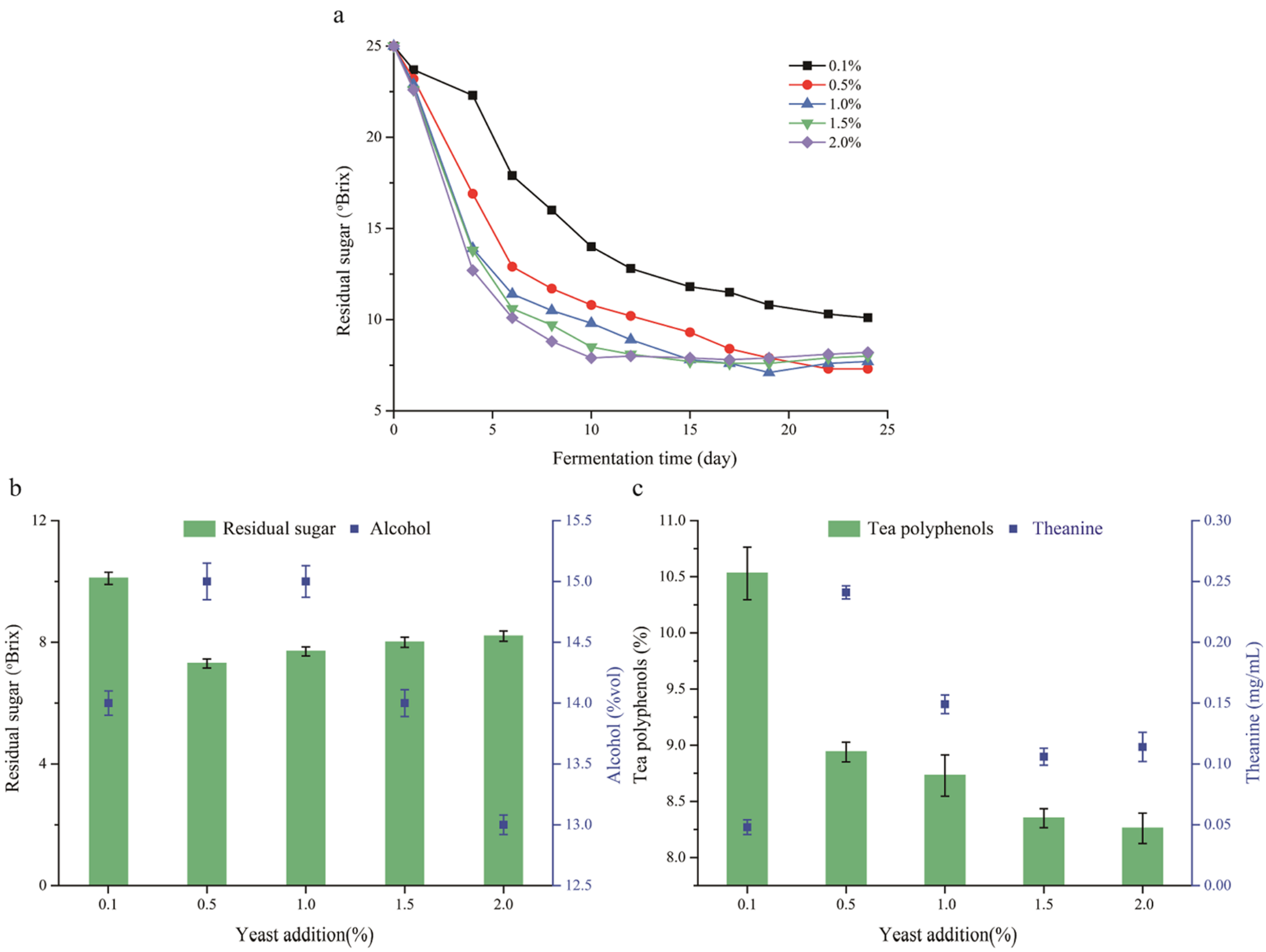

Figure 1. Effect of yeast addition on the quality of tea wine. Different yeast addition on the fermentation rate of tea wine (a), different yeast addition on residual sugars and the alcoholic content in tea wine (b), and different yeast addition on the content of tea polyphenols and theanine in tea wine (c).

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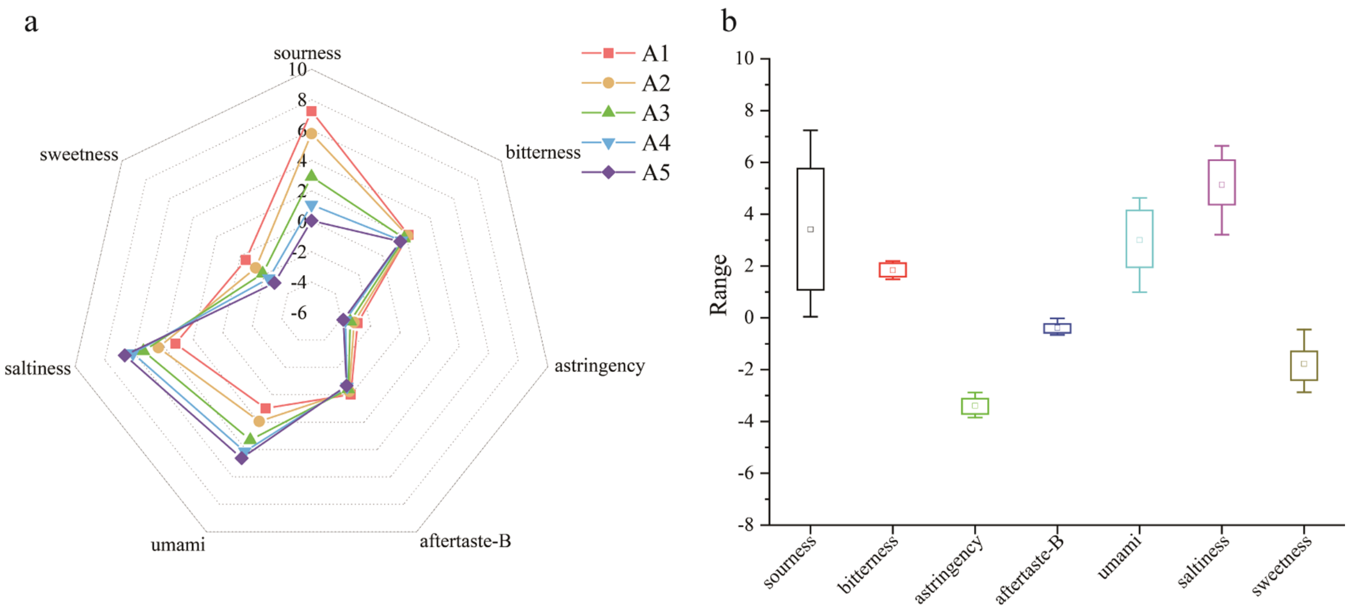

Figure 2. Electronic tongue analysis of the effect of yeast addition on the taste of tea wine. Radar chart of taste determination by the electronic tongue (a) and the taste detection value of the extreme difference analysis (b). A1−A5 means 0.1, 0.5, 1.0, 1.5, and 2.0% of yeast addition, respectively.

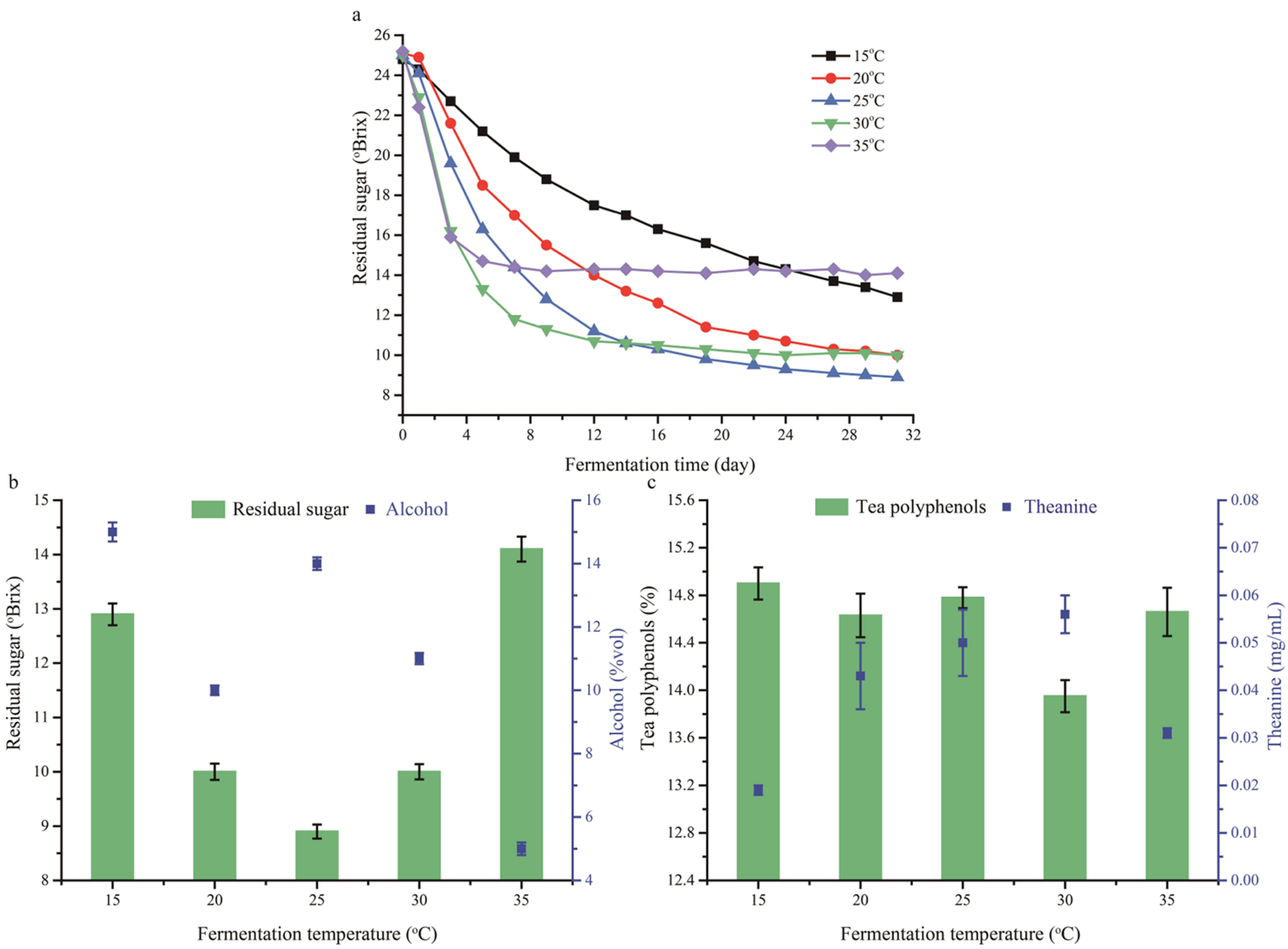

Figure 3. Effect of fermentation temperature on the quality of tea wine. Different fermentation temperatures on the fermentation rate of tea wine (a), different fermentation temperatures on residual sugars and alcoholic content in tea wine (b), and different fermentation temperatures on the content of tea polyphenols and theanine in tea wine (c).

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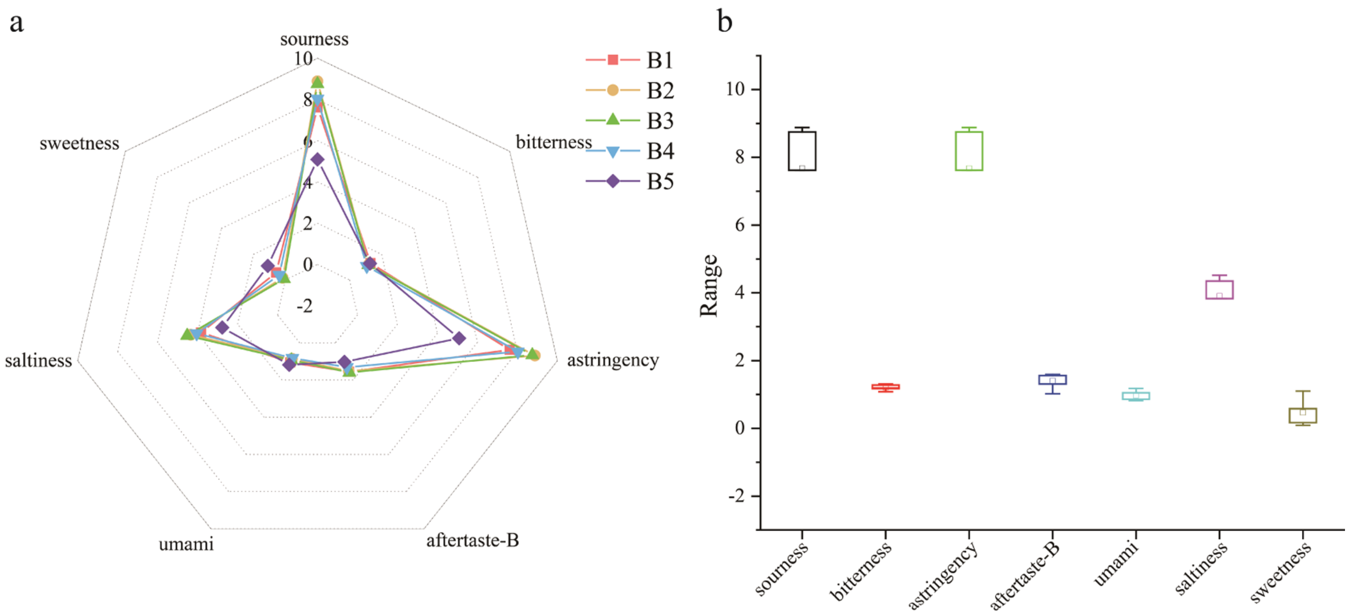

Figure 4. Electronic tongue analysis of the effect of fermentation temperature on the taste of tea wine. Radar chart of taste determination by the electronic tongue (a) and the taste detection value of the extreme difference analysis (b). B1−B5 means 15, 20, 25, 30, and 35 °C of fermentation temperature, respectively.

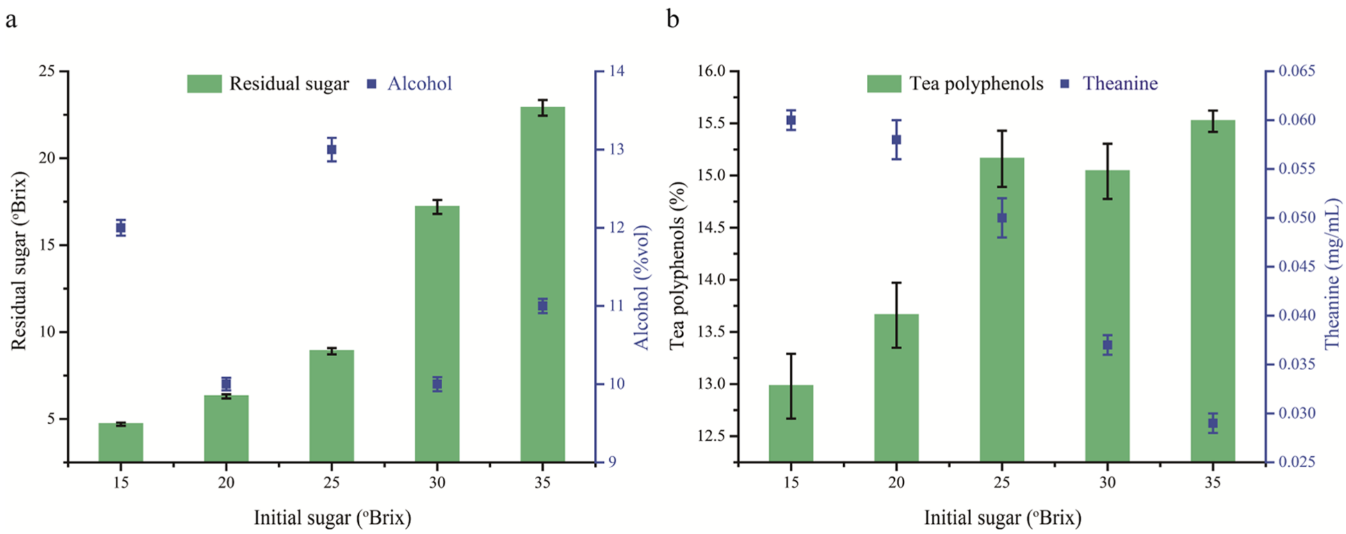

Figure 5. Effect of initial sugar on the quality of tea wine. Different initial sugar on residual sugar and alcoholic contents in tea wine (a) and different initial sugar on the contents of tea polyphenols and theanine in tea wine (b).

(BBD) was designed by Design-Expert 8.0.6 software to optimize the fermentation process of black tea wine.[14] The independent variables applied in the experimental design, such as yeast addition (0.1, 0.5 and 1.0%), initial sugar level (20, 25, and 30 °Brix), and fermentation temperature (20, 25, and 30 °C), were consistent with the coded levels (−1, 0, and 1), respectively (Table 1).

2.4. Electronic Setups and Signal Acquiring. The e-nose (PEN3, Airsense Analytics, Schwerin, Germany) used in this research is equipped with a metal oxide semiconductor (MOS) sensor array composed of 10 different MOS sensors. The performance of the sensor array to some specific volatile compounds is presented in Supporting Table S1. First, 5 mL of wine sample was collected in a 20 mL sample bottle, equilibrated at 25 °C (10 min), and then inserted into the e-nose probe for the detection. The specific parameters of the e-nose detection were 60 s detection time, 300 s cleaning time, 300 mL/min carrier gas speed, and 300 mL/min injection flow rate.[15]

An e-tongue (SA-402B, INSENT, Japan) was applied to detect the taste of black tea wine. The e-tongue comprises an automatic sampler, a sensor array, and signal processing software.[16] Briefly, 50 g of black tea wine was diluted with 150 mL of deionized water, and the supernatant was centrifuged at 4000 rpm for 10 min to be measured. Sensors were placed into the samples for 120 s to obtain the taste measurements.[17] After each taste measurement, the sensors were rinsed with distilled water for 10 s until reaching a stable potential to prevent cross contamination between samples.

2.5. Statistical Analysis. The experimental data were represented by mean value ± standard deviation (SD). Statistically significant differences ($P < 0.05$) were conducted

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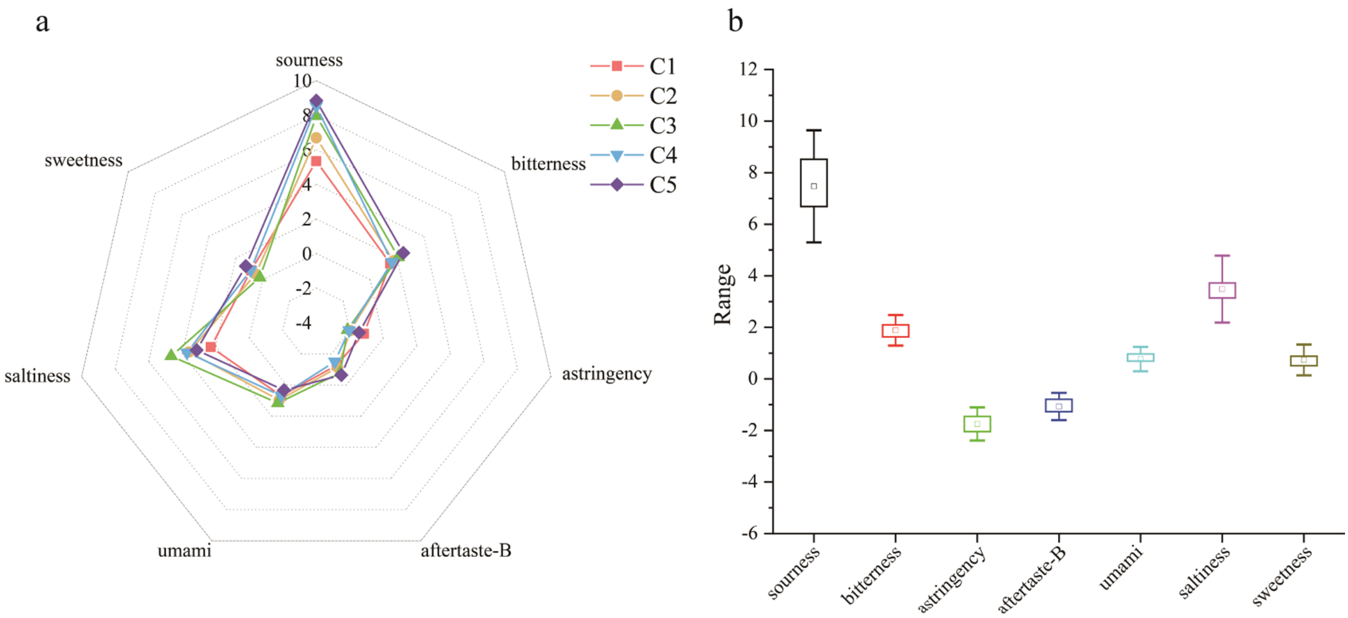

Figure 6. Electronic tongue analysis of the effect of initial sugar on the taste of tea wine. Radar chart of taste determination by the electronic tongue (a) and the taste detection value of the extreme difference analysis (b). C1−C5 means 15, 20, 25, 30, and 35 °Brix of initial sugar, respectively.

by variance (ANOVA) analysis using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL) and Origin version 9.1 (Origin Lab, Northampton, MA).

3. RESULTS AND DISCUSSION

3.1. Tea Wine Characteristics during Yeast Addition. As yeast is the key catalyst of tea wine fermentation, its biomass directly affects the process and quality of tea wine. Therefore, we investigated the effect of yeast inoculation on the tea wine quality in this study. With increasing yeast addition, the rate of wine formation accelerated, and the tea wine with 2.0% addition achieved its maximum alcoholic strength at the 10th day (Figure 1a). However, it showed the lowest alcoholic strength at 13.0% vol. The tea wine fermented with 0.5% yeast addition had the maximum alcohol (14% vol) and theanine (0.241 mg/mL) contents, respectively (Figure 1b,c). This is observed as a result of the gradual consumption of sugar by the yeast cells. This observation is similar to Ibegbulam et al.[18] and Teniola et al.[19] The gradual enhancement in the alcoholic content of carrot wine is observed as a result of the consumption of sugar by the yeast.[20] Evidence of sugar consumption by the yeast cells can be seen in the gradual reduction in specific gravity during the fermentation. The rate of sugar uptake by yeast cells has been monitored to be a consequence of the inherent kinetics of the transport process and substrate inhibition.[19,21]

The effect of yeast addition on tea wine taste by e-tongue is shown in Figure 2. The yeast addition had more impact on the tea wine acidity with extreme difference of up to 7.20, and the sourness showed a decreasing trend with the increase of the addition. Richness, bitter aftertaste, and astringent aftertaste had extreme difference values that were less than 1. It was reported that, even while differences existed between the two samples, they could not be distinguished sensorially if the relative intensities of the two samples had extreme difference values <1.[22] Figure 2 shows that the freshness and saltiness were positively correlated with the addition, indicating that when the addition increased, the tea wine's freshness and crispness were more prominent. Therefore, 0.5% yeast was finally determined at optimum addition.

3.2. Effect of Different Fermentation Temperatures. The effect of fermentation under different temperature conditions on tea wine quality was monitored. The rising temperature increased the fermentation rate, and the tea wine needed only 5 days at 35 °C for fermentation. In contrast, the residual sugar content was higher, and the alcohol yield was only 5.0% vol (Figure 3a). We hypothesize that there were increases in yeast growth and fermentation rates at 15−25 °C. Still, higher temperatures led to yeast mortality and lost fermentation viability, thus terminating fermentation. Similar results were found according to Beltran and Pham et al.[23,24] During 25 °C fermentation temperature, the alcohol (14% vol), tea polyphenol (14.78%), and theanine (0.05 mg/mL) contents showed moderate levels and the lowest residual sugar content (8.9 °Brix) at the end of fermentation (Figure 3b,c).

As shown in Figure 4, the temperature had more effect on tea wine sourness with an extreme difference value of 3.79. The bitterness and freshness were moderate, and the richness was maximum under 25 °C fermentation temperature. Therefore, 25 °C was determined as the optimum fermentation temperature.

3.3. Effect of Different Sugar Sources. Sugar sources act as precursors for alcohol synthesis and determine the alcoholic yield while providing carbon sources for yeast growth and facilitating the fermentation processes.[25] Therefore, the effect of different initial sugar levels on the tea wine quality was investigated in this study. The maximum alcoholic content was 13% vol when the content of initial sugar was 25 °Brix (Figure 5a), tea polyphenols was 15.16%, and theanine was 0.05 mg/mL (Figure 5b).

Figure 6 shows that the maximum difference in acidity caused by the initial sugar content in the tea wine, with an extreme difference value of 3.51 in acidity. However, there were no significant differences in bitterness, astringency, astringent aftertaste, and freshness caused by different initial sugar contents. The extreme difference values were <1, which

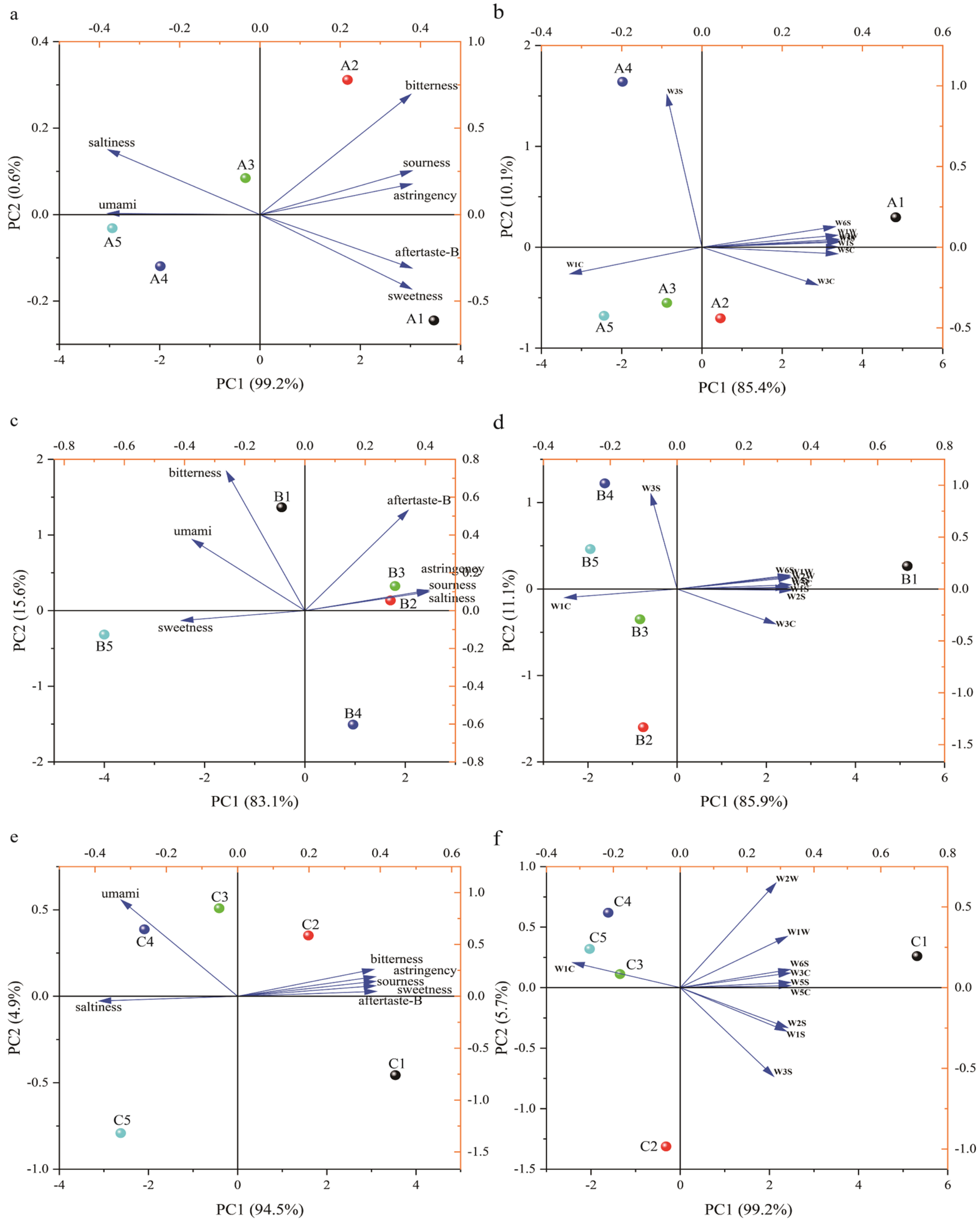

Figure 7. PCA of e-tongue and e-nose data of tea wine under different yeast addition (a, b), fermentation temperature (c, d), and initial sugar (e, f) conditions. A1−A5 indicates yeast addition (0.1, 0.5, 1.0, 1.5, and 2.0%), B1−B5 indicates fermentation temperature (15, 20, 25, 30, and 35 °C), and C1−C5 indicates initial sugar (15, 20, 25, 30, and 35 °Brix).

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Table 2. Summary Results of Box−Behnken Experimental Design

	factors			response value
run order	A	B	C	alcoholic content (% vol)
1	1	25	20	14.0
2	1	30	25	14.0
3	1	25	30	12.0
4	0.5	25	25	13.0
5	0.1	30	25	11.0
6	1	20	25	10.0
7	0.5	25	25	13.5
8	0.5	25	25	14.2
9	0.5	30	20	14.0
10	0.5	25	25	14.5
11	0.5	20	20	11.0
12	0.5	20	30	9.0
13	0.5	25	25	14.0
14	0.5	30	30	10.0
15	0.1	25	20	10.0
16	0.1	25	30	11.0
17	0.1	20	25	11.0

indicated no difference in sensory aspects. The optimum initial sugar content (25 °Brix) was determined.

3.4. Principal Component Analysis. The principal component analysis (PCA) is a well-known multivariate statistical method used to establish a biunivocal description of the samples in terms of experimental coordinates.[26] Therefore, this study analyzed the flavor evaluation of tea wine by an electronic tongue and electronic nose using principal component analysis. According to Figure 7, the principal components of the e-tongue contributed 99.8, 98.7, and 99.4% of the total variance for yeast addition, fermentation temperature, and initial sugar content, respectively. These values were similar to those of the e-nose, with a total variance of 95.5, 97.0, and 97.9%, respectively. The researchers showed that more than 85% of the total variance indicated a rigorous classification procedure. The higher total variance and more principal component can reflect the information.[9,27] Therefore, the results of PCA showed that the data from the e-tongue and e-nose were stable and could be used as a basis for analysis.

An e-tongue is a novel artificial taste recognition technology widely applied in food and beverage evaluation due to its advantages, such as fast response and no fatigue problems.[28,29] Bitter, sour, astringent, aftertaste-B, and sweet flavors contributed similarly to the PC1 axis under the influence of yeast addition. They could be distinguished from savory and fresh flavors (Figure 7a). Astringent, sour, salty, and aftertaste-B flavors showed positive correlations with the PC1 axis under the influence of fermentation temperature. In contrast, bitter, fresh, and sweet flavors exhibited negative associations (Figure 7c). Figure 7e shows that the savory and fresh flavors of the tea wine differed significantly from the other flavors under the influence of the initial sugar level. The differences were mainly reflected in the PC1 axis.

An e-nose is sensitive to the odor of the samples, and slight changes in the composition of volatile compounds may result in a different sensor response.[27] Figure 7b,d,f shows the PCA of volatile compounds in tea wine under various fermentation conditions. Samples 1, 2, 3, 4, and 5 were well distinguished from each other, indicating the significantly different volatile components presented in these eight soybean meal hydrolysates. Alcohols, aldehydes, ketones, and alkanes contributed to volatiles under the influence of yeast addition and fermentation temperature. In contrast, nitrogen oxides, aromatics, and organic sulfides contributed under the influence of the initial sugar content.

3.5. Response Surface Methodology Model for Alcohol Content. As a major parameter for fermented wine, the alcohol content plays an essential role in the traditional alcoholic fermentation process.[30] Therefore, based on the results of single-factor experiments, a three-factor, three-level Box−Behnken response surface experiment was conducted for yeast addition (A), initial sugar (B), and fermentation temperature (C) in this study, using alcoholic strength as the response value. Table 2 provides experimental data for the tea wine fermentation results examined under different fermentation conditions. Table 3 shows the variance analysis for the regression response surface methodology (RSM) model.

The regression equation is shown in eq 1.

$$Y = 13.95 + 0.88A + 1.06B - 0.91C + 0.99AB - 0.68AC - 0.50BC - 0.90A^2 - 1.55B^2 - 1.29C^2, \ R^2 = 0.889 \quad (1)$$

Table 3. Analysis of Variance (ANOVA) for the Response Surface

source	sum of squares	degree of freedom	mean of square	*F*-value	*p*-value	significance
model	48.96	9	5.44	6.25	0.0123	[a]
A	6.13	1	6.13	7.03	0.0328	[a]
B	8.85	1	8.85	10.17	0.0153	[a]
C	6.62	1	6.62	7.6	0.0282	[a]
AB	3.98	1	3.98	4.57	0.07	ns
AC	1.84	1	1.84	2.11	0.1892	ns
BC	1	1	1	1.15	0.3194	ns
A^2	3.33	1	3.33	3.82	0.0914	ns
B^2	10.05	1	10.05	11.54	0.0115	[a]
C^2	7.06	1	7.06	8.11	0.0248	[a]
residual	6.1	7	0.87			
probability	4.68	3	1.56	4.42	0.0925	ns
SE	1.41	4	0.35			
total	55.06	16				

[a]Note: ns, indicating the significance level as $0.01 < p < 0.05$, not significant.

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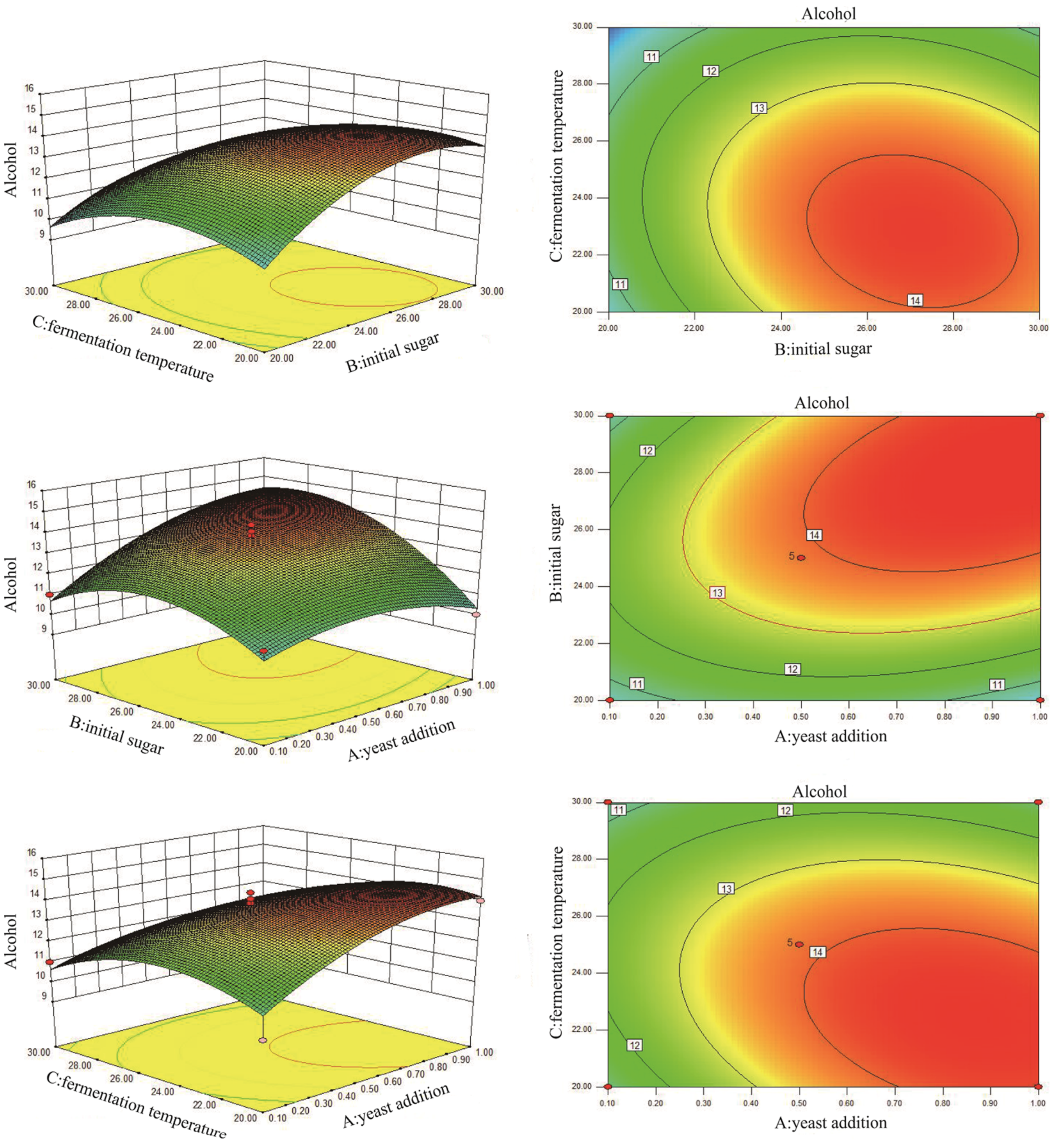

Figure 8. Response surface for the effect of independent variables on alcohol content.

The probability of associated *p*-values of 0.0925 showed nonsignificance of difference, demonstrating that the model fits with the data.[13,14] Based on the *F*-value results, the effect of the experimental factors on the alcohol content was initial sugar (*B*) > fermentation temperature (*C*) > yeast addition (*A*). In the selected levels, the one-time item yeast addition (*A*), initial sugar (*B*), fermentation temperature (*C*), and quadratic term B^2 and C^2 were significant ($p < 0.05$). The three-dimensional response surfaces showed the relationships between independent and dependent variables.[31] The steeper the surface plot, the more significant the interaction between the variables.[32] Response surface plots showing the effect of independent variables on the alcoholic content of the tea wine are shown in Figure 8. The surfaces tended to level off and combined with the ANOVA indicated that the interaction between the variables was not significant.

The ideal fermentation parameters for tea wine are initial sugar content (25 °Brix), yeast addition (0.5%), and fermentation temperature (25 °C), according to the statistical software Design-Expert V8.0.6′s model optimization solution.

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To verify the reliability of the predicted value, a practical verification test was conducted under these conditions. The actual measured alcoholic content of the tea wine was 14.0% vol, which was similar to the predicted value (13.9% vol). It can be seen that the predicted values of the indicators were in better agreement with the experimental values, which further demonstrated that the model could accurately predict the experimental results.

4. CONCLUSIONS

Electronic noses and tongues consist of a series of nonspecific sensors with cross-sensitivity that respond to a large number of compounds. Validation is also a problem in e-noses and e-tongues. In the case of wines, the problem of weak validation is particularly important because wines change with time, and quality depends on various conditions, i.e., weather. The optimal conditions for the fermentation process were determined as the initial sugar content (25 °Brix), yeast addition (0.5%), and fermentation temperature (25 °C) in the present study, which used RSM to optimize the key factors of black tea wine fermentation. The model predicted 13.9% vol alcohol for tea wine under these conditions. The validation experiment yielded 14.0% vol alcohol, 0.05 mg/L theanine, and 15.16% tea polyphenol, 22.0 and 716.4% enhanced from the initial tea wine. The black tea wine produced under these conditions has an orange-red color, full wine and tea flavor, and mild and mellow taste. The analysis of the electronic evaluation system obtained that all three fermentation factors had the greatest influence on the sourness of the black tea wine. Yeast addition and fermentation temperature made the maximum contribution of alcohols, aldehydes, and alkanes volatiles, while the initial sugar content made the maximum contribution of nitrogen oxides, aromatics, and organic sulfides to volatile components. In future work, more samples are needed to screen fewer independent variables to make the prediction equation more accurate and stable. In addition, we will focus on the aroma, color, and taste of tea wine by introducing the e-nose- and e-tongue-based techniques, with the aim to provide all round data support for comprehensive quality assessment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00862.

Electronic nose sensor properties (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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