

Beef tallow enzymatic hydrolysis and flavoring process based on BP neural convolution and GC-MS fingerprinting

Zien Li ^{1, a}, Shuangshuang Cheng ^{1, b}, Xinyu Wang ^{1, c}, Lei Zhang ^{1, d}, Yanfei Yang ^{1, e}, Tianxue Sun ^{1, f}, Yanxia Xing ^{1, g}, He Zhu ^{1, h} and Chunlin Liu ^{2, *}

¹ Shandong Agricultural Engineering Institute, Shandong, China

² Shandong Institute for Food and Drug Control, Shandong, China

*Corresponding author e-mail: 475046094@qq.com, ^aqtwqwqtw0731@qq.com,

^b1794128038@qq.com, ^c534045539@qq.com, ^d2764726379@qq.com,

^e1817515421@qq.com, ^f3365717699@qq.com, ^g603313136@qq.com, ^h 251763119@qq.com

Abstract

This study aims to investigate the impact of lipase on enhancing the flavor of beef tallow, focusing on the influence of key parameters including time, temperature, and enzyme dosage on its sensory properties. A BP neural network-based model is developed to predict the sensory quality of beef tallow. Additionally, to delve deeper into the alterations in flavor components induced by lipase, the volatile compounds in beef tallow, both pre- and post-enzymatic treatment, are analyzed using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). Key volatile components are identified based on their relative abundance and subjected to principal component analysis. A comprehensive scoring system is formulated to assess the quality of beef tallow quantitatively. Findings reveals that the optimal conditions for lipase treatment to enhance beef tallow flavor were a temperature of 37 °C, a duration of 80 minutes, and an enzyme dosage of 0.04 mg/g. The BP neural network model demonstrates a high fitting accuracy of 0.978 for predicting the decomposition of beef tallow by lipase. Post-treatment analysis indicates a significant reduction in aldehydes and ketones ($P < 0.05$) and a notable increase in alcohols ($P < 0.05$), culminating in the highest comprehensive score of 8.22, surpassing that of the untreated sample. These findings not only offer a scientific foundation for optimizing beef tallow flavor but also open new avenues for the application of lipase in oil processing. This research lays a crucial groundwork for the industrial production of enhanced beef tallow, highlighting its significant theoretical and practical implications.

Keywords

BPcnn, enzyme, flavor

1. Introduction

Beef tallow flavor is a very important quality criterion and the main indicator that determines its popularity in the market. Beef tallow itself does not have a fragrance, and its fragrance is mainly produced by the oxidative degradation of lipids during the heating process, and the substances produced are divided into two parts, one part is directly flavored as a fragrance substance, and the other part can be used as a reaction precursor to participate in the competition Maillard reaction for fragrance. In the beef tallow refining process, the lack of flavor is a common problem. The methods of increasing the flavor of beef tallow mainly include fermentation, adding flavors and fragrances, enzymatic hydrolysis, etc., among which the lipase decomposition of beef tallow flavor enhancement technology stands out because of its significant advantages. Lipase can decompose triglycerides in beef tallow into fatty acids,

methyl ketones and lactones, which can be further oxidized and decomposed into small molecules of aldehydes and ketones [1], thereby significantly improving the flavor richness of beef tallow. Moreover, the process of lipase decomposition of beef tallow is environmentally friendly and pollution-free, which is an ideal method for flavoring at present. However, during the sensory evaluation of enzyme-modified beef tallow, the results can be easily influenced by the evaluator's mindset, environmental factors, as well as personal experiences and preferences, leading to unstable and difficult-to-quantify results. Mahdi Ghasemi-Varnamkhasti et al.[2] conducted sensory quality predictions using beef as the material based on a BP neural network, and the results indicated a good fit of the neural network prediction model. Similarly, Qingli Dong[3] used cooked sausage as the material for sensory predictions based on a BP neural network, showing a high goodness-of-fit and low error in their prediction model. Therefore, the construction of a BP neural network model is relatively reliable. Currently, there is still a limited amount of research on sensory evaluation of beef tallow using BP neural network models. This study uses enzyme-modified beef tallow as the raw material and, based on its sensory assessment, employs a BP neural network to construct a sensory evaluation model for beef tallow. Additionally, solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) technology is utilized to determine and analyze the volatile components in the beef tallow before and after the addition of lipase, providing a scientific basis for the optimization of beef tallow flavor.

1.1. Materials and Instruments

1.1.1. Preparation of raw materials.

beef tallow [peroxide value 0.020 g/100 g], Shandong Shangdu Heng chang Animal Husbandry Co. Ltd, cryopreserved at -18°C; Freshly refined beef tallow is liquid, yellowish in color, can solidify into a solid at room temperature, has a special aroma, and is stored at -18°C for later use; Lipase, Shanghai Xuan yuan Bioengineering Co., Ltd., enzyme activity unit: 500 u/g.

1.1.2. Experimental reagents and instruments.

Sodium thiosulfate, Tianjin Kemiou Chemical Reagent Co, Ltd; Potassium iodate, Sinopharm Chemical Reagent Co., Ltd; Potassium iodide, Tianjin Beilian Fine Chemicals Development Co., Ltd; Glacial acetic acid, Wuxi Jinko Chemical Co., Ltd; Cyclohexane, Tianjin Kemiou Chemical Reagent Co., Ltd; Wechsler Reagent, Tianjin Kemiou Chemical Reagent Co., Ltd.; Sulfuric acid, Yantai Far East Fine Chemical Co., Ltd; Sodium carbonate, Tianjin Kemiou Chemical Reagent Co., Ltd; Soluble starch, Xilong Science Co., Ltd.

Electronic Analytical Balances, METTLER TOLEDO Instruments Ltd; Collector constant temperature heating magnetic stirrer, Shanghai Lichen Bangxi Instrument Co., Ltd; Digital display constant temperature water bath, Shanghai Chengjie Instrument Equipment Co., Ltd; Meat grinder, Hunan Yangzi Intelligent Technology Co., Ltd; Gas-mass spectrometry analyzer, Agilent; Constant temperature bath, Gongyi Yingyu Hi-Tech Instrument Factory; 75µm PDMS/CAR Fiber Extraction Fiber, Beijing Kanglin Technology Co., Ltd.

1.2. Experimental Methods

1.2.1. Enzymatic hydrolysis of beef tallow flavoring process

Split the oil→Fascia removal→Rinse→Chop into cubes and stir up→Refining→Lipase was added to adjust the pH value and the reaction was carried out after 7~8→Enzyme inactivation(85°C water bath for 30min)

1.2.2. Enzymatic hydrolysis of beef tallow single factor assay.

In the process of enzymatic hydrolysis, the main factors affecting the sensory characteristics of the enzymatic hydrolysis reaction are enzymatic hydrolysis temperature, enzymatic hydrolysis time, enzyme dosage, etc. Therefore, this factor was used to conduct a single factor experiment,

and 20 sensory evaluators were selected to evaluate the sensory evaluation of enzymatically hydrolyzed beef tallow.

Selection of one-factor ranges. When selecting the temperature, too high temperature will lead to inactivation of lipase, and too low temperature will lead to a decrease in enzyme activity and insufficient reaction, so the temperature should be selected in a suitable range to make it have a good enzymatic hydrolysis effect. The enzymatic hydrolysis temperature was set to 35 °C, 37 °C, 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C, and the three groups of parallel experiments were carried out and the sensory scores were averaged.

When the time was selected, the enzymatic hydrolysis time was set to 20 min, 40 min, 60 min, 80 min, 100 min, and 120 min, and the three groups of parallel experiments were carried out and the sensory scores were averaged.

The appropriate range should also be selected for the selection of enzyme dosage, and the enzyme dosage should be set to 0.02 mg/g, 0.04 mg/g, 0.06 mg/g, 0.08 mg/g, and 0.1 mg/g. Three sets of parallel experiments were carried out and the sensory scores were averaged, and each optimal choice was taken as the fixed value of the next item, and a blank control was designed to select the optimal combination.

Enzymatic hydrolysis of beef tallow orthogonal experiment. Based on the results of the single factor experiment in 2.2.2, the L9(3³) experimental design was carried out, and the specific factor levels are shown in Table 1:

Table 1. Orthogonal experimental factor levels

level	A temperature °C	B Time min	C Add the amount of enzyme mg/g
1	37 °C	60 min	0.02 mg/g
2	40 °C	80 min	0.04 mg/g
3	45 °C	100 min	0.06 mg/g

1.2.3. Sensory scoring criteria.

Referring to the sensory requirements of GB 10146-2015 "National Food Safety Standard for Edible Animal Fats", based on the sensory results of pure beef tallow, the sensory scoring criteria for enzymatic hydrolysis of beef tallow (see Table 2 below) were formulated, and 10 sensory evaluation personnel engaged in food research were selected, and a sensory evaluation team was formed according to the sensory scoring standards, and the samples were cooled to room temperature and then heated to the selected temperature, and then sensory evaluation was carried out in the odorless sensory analysis room. 10 points overall.

Table 2. Sensory scoring criteria

index	standard
smell	Oily and fragrant, no smell, rancidity (3~4); The oil taste is light, there is a bad smell, and the rancid taste (1~3); No oily smell, with bad smell and rancid smell (0~1)
Color	Golden (2~3); light yellow (1~2); White(0~1)
morphology	Liquid (2~3) Semi-solid (1~2) Solid (0~1)

1.2.4. Establishment of BP neural network

Introduction to BP Neural Networks. The BP algorithm uses the backpropagation mechanism to iteratively optimize the network weights and biases by inputting learning samples[4], and gradually adjusts the network parameters by minimizing the difference between the output vector and the expected vector. When the sum of the squares of the errors of the output layer

of the network is less than the specified error, the training process is terminated and the model reaches a convergent state. Linear models are often difficult to deal with nonlinear problems, so it is necessary to refer to the activation function to delinearize the neural network, and the BP neural network uses the Sigmoid function, which is divided into an input layer, a hidden layer, and an output layer, and its propagation mode is shown in Figure 1:

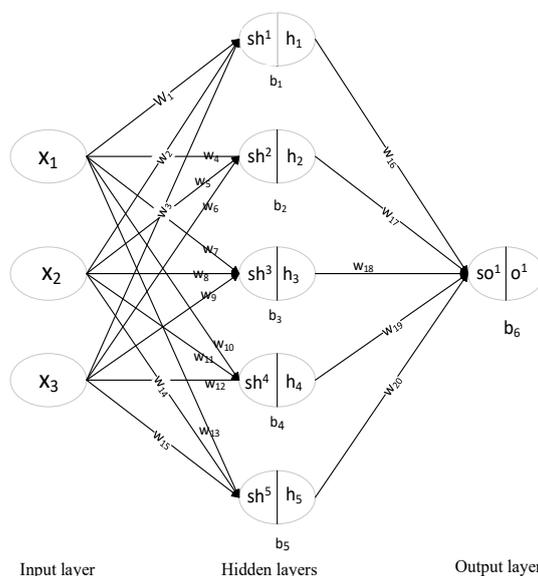


Figure 1 BP neural network propagation mode

Matlab creates BP neural networks. Due to the complexity of neural networks, the large amount of data computation, and the large amount of manual computing data, which are difficult to calculate in a short time, the establishment and calculation of neural networks by programming greatly enhances the computational efficiency[5]. Therefore, MatlabR2023b programming is used to build and compute neural networks. In this paper, temperature, time, and enzyme dosage data were used as the input layer[6], and sensory scores were used as the output layer to predict the senses. The training was performed using a strategy called "early termination", in which 18 sets of data were randomly selected as the training set and 9 groups were selected as the test set for BP neural network training on a total of 27 sets of univariate and orthogonal data[5,7].

1.2.5. Determination index and method of iodine value of beef tallow.

The iodine value was determined with reference to the "Determination of Iodine Value of Animal and Vegetable Oils and Fats" (GB/T 5532-2022).

Calculated by formula:

$$W = \frac{(V_1 - V_2) \times C \times 12.69}{m}$$

where:

W — The iodine value, i.e., the number of grams of iodine absorbed per 100 g of sample, is measured in grams per 100 grams(g/100 g);

*V*₁ — The blank solution consumes the volume of sodium thiosulfate standard solution in milliliters(mL);

*V*₂ — The sample solution consumes the volume of sodium thiosulfate standard solution in milliliters(mL);

C — The concentration of sodium thiosulfate standard solution in moles per liter(mol/L);

m —The mass of the specimen, in grams(g)

1.2.6. Determination of volatile components of beef tallow.

Determination of volatile components by solid-phase microextraction coupled with gas chromatography-mass spectrometry (GC-MS) was performed on beef tallow with different treatments. Weigh 2 g of each sample and place it in a sample vial. The specific parameters are as follows:

The conditions for microextraction were as follows: extraction temperature 60 °C, extraction time 30 min. Instrument Condition: HP-5MS Capillary Column (19091s-436; 325 °C ; 60 m × 0.25 mm × 0.25 μm), equilibrate on the fiber for 1 h.

Chromatographic conditions: Modified according to the experimental conditions of A Sharma et al.[8] HP-5MS capillary chromatography column was used, high-purity helium was used as the carrier gas, the injection port temperature was 250 °C, the flow rate was 1mL/min, and the split ratio was 4:1. Heating program: Start at 40 °C, hold for 1 min, and heat up to 250 °C at a heating rate of 5 °C/min for 5 minutes.

Mass spectrometry conditions: electron bombardment (EI) ion source energy 70 ev, ion source temperature 230 °C, transmission line temperature 250 °C, scanning range m/z 35~350

Qualitative and quantitative analysis. Qualitative analysis: The volatile flavor compounds were identified in the NIST 14.L library, and the flavor compounds with a matching degree of > 80% were selected for qualitative analysis. Quantitative analysis: Area normalization and quantification were used to quantitatively analyze each component.

Data processing. The orthogonal design test assistant and Origin 2022 software were used for data collation and plotting. SPSS 25.0 software was used for statistical analysis, and the results were expressed as mean ± standard deviation (means ± SD). The experimental data were analyzed by one-way ANOVA and Duncan multiple comparisons[9], and the difference was significantly expressed with a $P < 0.05$.

2. Results and Analysis

2.1. Univariate outcome analysis

2.1.1. Determination of digestion temperature

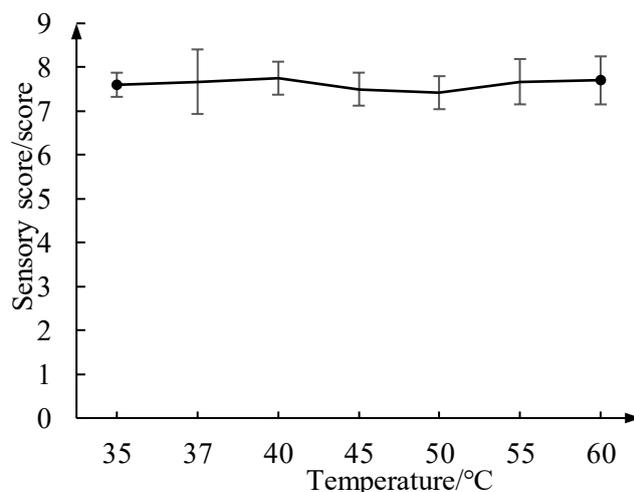


Figure 2 Effect of temperature on the senses

It can be seen from Figure 2 that with the increase of temperature, the sensory score first increased and then decreased, but the overall change was not large, and it was found that the enzymatic hydrolysis temperature had an effect on the sensory properties of beef tallow, and

the sensory score of beef tallow was the highest at 40°C, and the lowest sensory score at 50°C ($P < 0.05$). At 37°C~45°C, the score is relatively high, with a strong aroma and good flavor.

2.1.2. Determination of digestion time

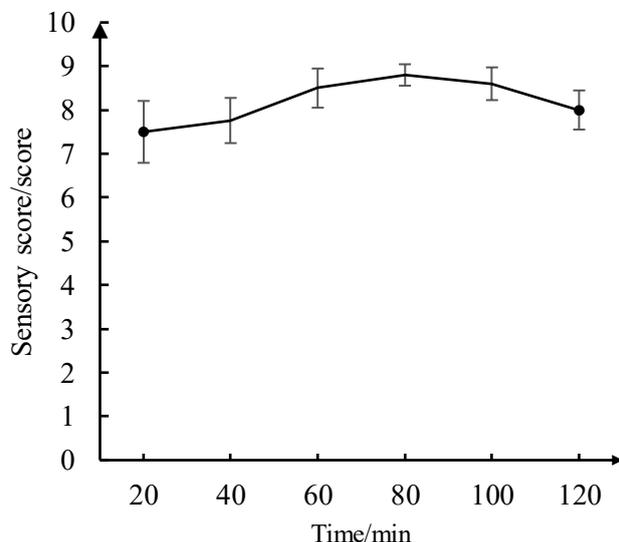


Figure 3. Effect of time on the senses

It can be seen from Fig. 3 that with the increase of time, the sensory score first increased and then decreased, reaching the highest score of 8.8 at 80 min and the lowest score at 20 min, with a significant difference ($P < 0.05$). When the enzymatic hydrolysis time is 60~100, the score is relatively high, and the flavor is better, and the taste is too heavy if the time is too long.

2.1.3. Determination of the amount of enzyme to be added.

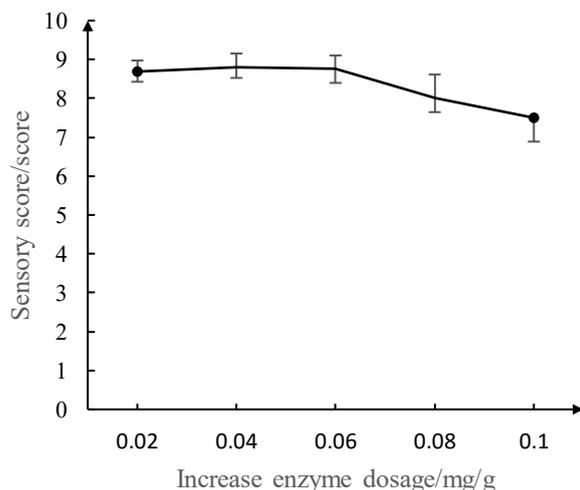


Figure 4. Effect of enzyme dosage on the senses

It can be seen from Figure 4 that with the increase of enzyme dosage, the sensory score first increased and then decreased, reaching the highest score of 8.8 at 0.04 mg/g and the lowest at 0.01 mg/g, with a significant difference ($P < 0.05$). At 0.02 mg/g~0.06 mg/g, the score was relatively high, with a significant difference ($P < 0.05$), and the oily smell was aggravated after increasing the enzyme dosage, resulting in discomfort.

2.2. Orthogonal results analysis

Table 3. Orthogonal experimental results

Experiment serial number	Factor			Sensory scores
	A temperature °C	B Time min	C Add the amount of enzyme mg/g	
1	1	1	1	8.67
2	1	2	2	8.94
3	1	3	3	8
4	2	1	2	8.6
5	2	2	3	8.5
6	2	3	1	8.47
7	3	1	3	8
8	3	2	1	8.5
9	3	3	2	8.4
K1	25.61	25.27	25.64	
K2	25.57	25.94	25.94	
K3	24.9	24.87	24.5	
k1	8.54	8.42	8.55	
k2	8.52	8.65	8.65	
k3	8.3	8.29	8.17	
R	0.24	0.36	0.48	C>B>A

The orthogonal experimental results in Table 3 show that the order of action of each factor is C>B>A, and the relationship between the level values of each factor is as follows: A1> A2>A3; B2>B1>B3; C2> C1>C3, comprehensive k value, and intuitive comparison, the optimal combination A1B2C2 was obtained. Therefore, it can be concluded that the optimal conditions for the effect of lipolytic enzyme on the flavor of beef tallow were as follows: the action temperature was 37 °C, the action time was 80 min, and the enzyme dosage was 0.04 mg/g.

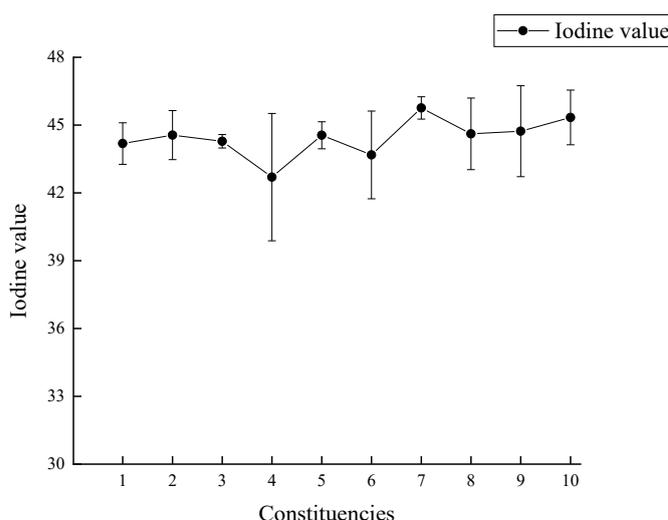


Figure 5. Chart of changes in iodine value of beef tallow

2.3. beef tallow iodine value analysis.

As can be seen from Figure 5, categories 1-9 are serial numbers 1-9 in orthogonal experiments, and category 10 is beef tallow after refining. Compared with the refined beef tallow, most of the

iodine value of the beef tallow after the enzyme reaction is significantly reduced, which means that the saturated fatty acids obtained by the reaction after the enzyme are added, and the degree of oxidation and the content of saturated fatty acids produced are also different due to the different content, temperature and time of the enzyme. The decrease of iodine value and the increase of fatty acids also affected the sensory evaluation of beef tallow.

2.4. Analysis of BP neural network results

2.4.1. Hide layer selection.

The number of hidden layers will also have a great impact on the neural network, too low will lead to the inability to accurately calculate the relationship between the conditions and the results, too low will also lead to a long time, the amount of computation is huge, according to the empirical formula: the number of hidden layers = a can be obtained The number of hidden layers is 1~10=3~12, subject to the mean square error, the hidden layer 3~12 is selected for multiple training, and the lowest is selected for comparison. As shown in Table 4, the mean square error is the smallest when the hidden layer is 5, which is 0.0025, and Figure 6 is the mean square error plot of the hidden layer of 5. Therefore, a neural network with a hidden layer of 5 is chosen. (The smaller the mean square error, the better the effect).

Table 4. Select the number of hidden layers

The number of layers is hidden	Mean square error	The number of layers is hidden	Mean square error
3	0.05	8	0.033
4	0.04	9	0.009
5	0.0025	10	0.006
6	0.003	11	0.014
7	0.02	12	0.124

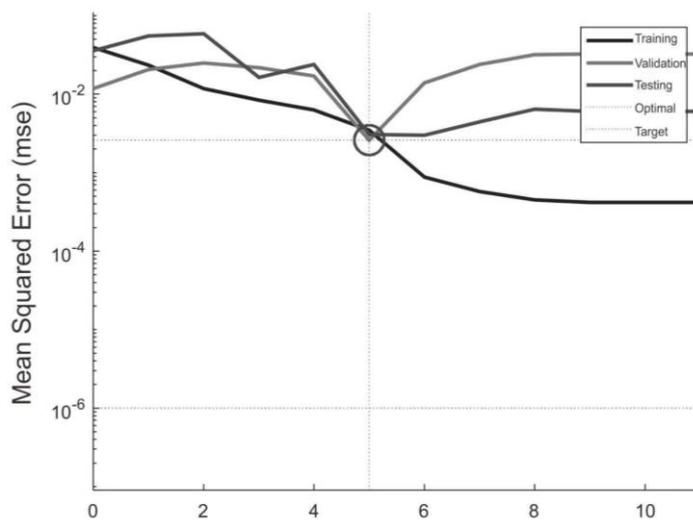


Figure 6. Mean square error results

2.4.2. Fit analysis.

As shown in Figure 7, the circles represent the discrete error between the actual value of the data and the predicted value, the closer the data is to the fitting line, the smaller the error between the predicted value and the actual value[10], as shown in the figure above, it can be seen that the fitting degree R of the training set is 0.975, the fitting degree R of the test set is 0.995, the fitting degree R of the validation set is 0.986, and the fitting degree R of the total

sample is 0.978, and the condition that the model does not have an overfitting state condition: R test set is >The R value of the R validation set, the validation set and all datasets are greater than 0.9, and the model does not have an underfitting state. Therefore, the model has a good prediction effect, and the optimal combination is 37°C, 80 min, and 0.04 mg/g.

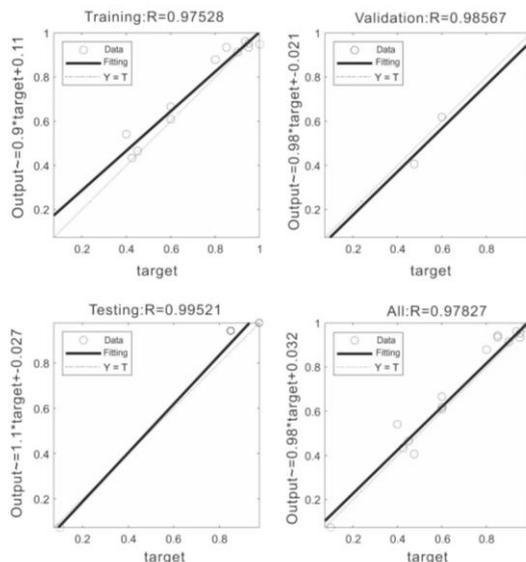


Figure 7. Data fitting results

2.4.3. Comparison of predicted and expected values is analyzed.

As can be seen from Figure 8, the asterisk represents the true value and the circle represents the predicted value, which can intuitively reflect the comparison between the true value and the predicted value, and the real value (action temperature 37 °C, action time 80 min, enzyme addition 0.04 mg/g, total sensory score 8.94) is in good agreement with the predicted value (total sensory score 8.79), although there is a certain error and the maximum error is 2.9%, but there is good volatility and high accuracy. For the entire hydrolysis process, the predicted values obtained by the BP neural network model achieve a good fit to the original sample values.

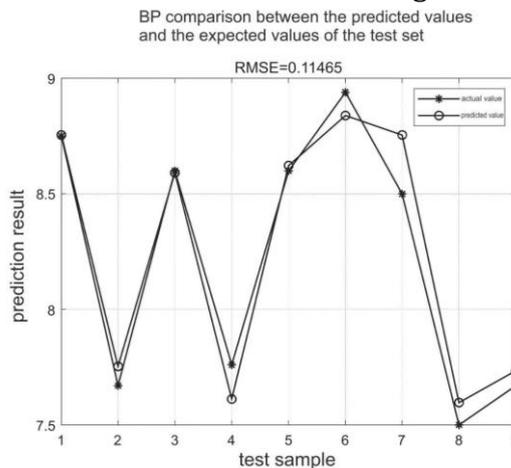


Figure 8. Comparison of the predicted and actual values of the test sample

2.5. Analysis of volatile flavor components of beef tallow

2.5.1. Analysis of volatile flavor components and relative content of beef tallow.

The total ion chromatography of the volatile components of beef tallow is shown in Figure 9, and the types of volatile components and their percentages in the total are shown in Table 5.

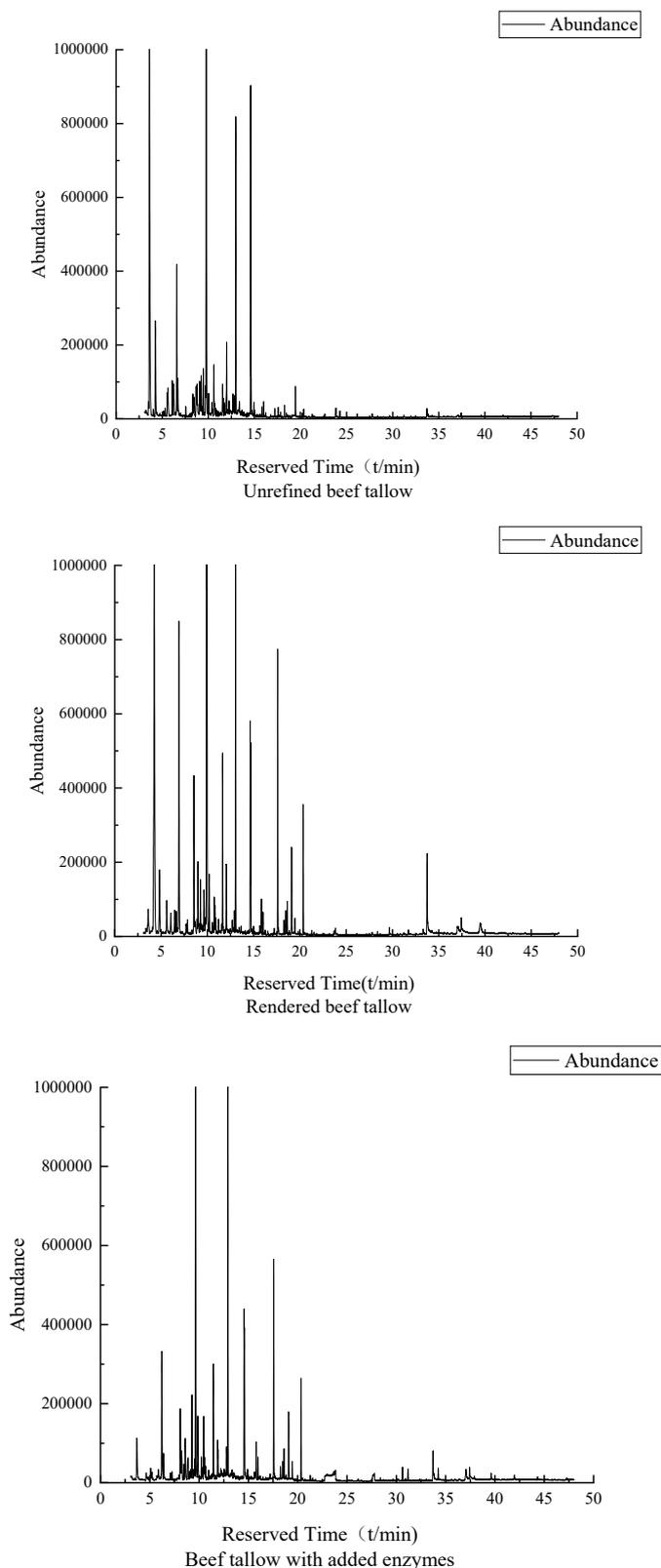


Figure 9. Total ion current plot of shea beef tallow volatile components GC-MS

Table 5. Types of volatile components and their percentages in different treatments

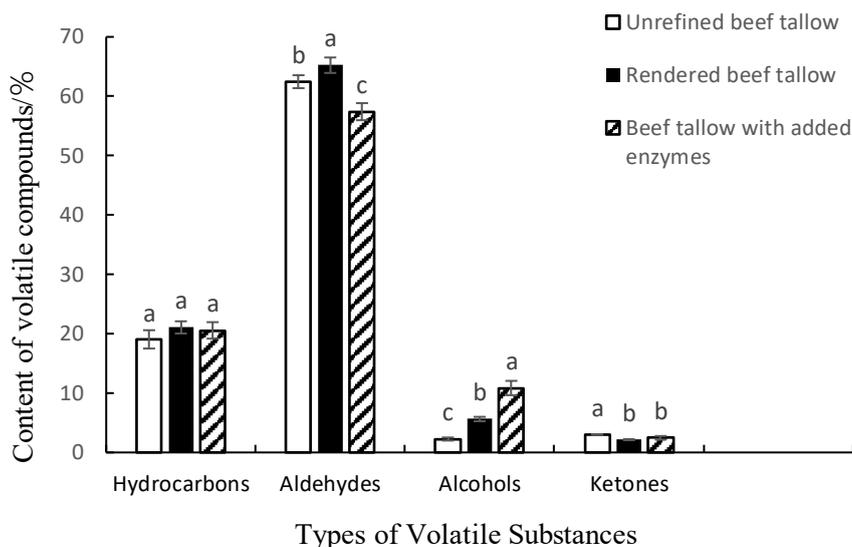
volatility ingredients	Unrefined beef tallow		beef tallow after refining		beef tallow after adding enzymes	
	type	Percentage/%	type	Percentage/%	type	Percentage/%

Hydrocarbons	19	19.05	12	21.07	17	20.56
Aldehydes	11	62.45	14	65.23	10	57.40
Ketones	3	3.03	8	2.15	2	0.99
Acids	3	1.13	3	2.71	2	2.47
alcohols	2	2.26	8	5.63	10	10.85
Esters	6	4.83	2	0.35	1	0.49
Ethers	2	0.58	1	0.27	0	0
Phenols	1	0.17	0	0	0	0
Heterocycles	7	6.50	5	2.59	6	7.24
as well as other classes						
sum		54		53		48

It can be seen from Table 5 that there were differences in the composition and content of volatile components in different treatments, and 54, 53 and 48 volatile flavor components were identified in unrefined oil, refined oil and oil after refining with lipase reaction, respectively. From the perspective of categories, the content of aldehydes and hydrocarbons accounted for the largest proportion of the total categories, accounting for more than 70% of the sum of the three beef tallows. Among them, the proportion of beef tallow hydrocarbons after refining was the highest at 21.07%, and the proportion of unrefined beef tallow hydrocarbons was the lowest at 19.05%. The highest proportion of aldehyde after refining was 65.23%, and the lowest proportion of beef tallow after enzyme was 57.40%.

2.5.2. Analysis of volatile flavor components in beef tallow.

As can be seen from Figure 10, aldehydes are the main flavor compounds of beef tallow, and the threshold is low, which contributes the most to the overall flavor of beef tallow. After refining, the content of aldehydes in beef tallow increased significantly ($P < 0.05$), and with the increase of melting temperature, the automatic oxidation rate of fatty acids accelerated, resulting in a significant increase in the types and contents of aldehydes[11], and the fat in beef tallow underwent hydrolysis and oxidation reactions at high temperatures. Fatty acids are first converted into hydroperoxides during the oxidation process, and these hydroperoxides are further decomposed to produce secondary oxidation products such as aldehydes, alcohols, and acids. At the same time, the fat oxidation process also induces protein oxidation, and then produces volatile flavor substances such as aldehydes and ketones[12], and the content of shea beef tallow substances also increased significantly after refining ($P < 0.05$), and the content of shea aldehydes decreased significantly after adding enzymes ($P < 0.05$), while the content of alcohols increased significantly ($P < 0.05$), which may be due to the decomposition of triglycerides into glycerol and fatty acids, including saturated fatty acids and unsaturated fatty acids, in the enzymatic reaction[13]. Since aldehydes mainly come from the oxidation of unsaturated fatty acids, and the iodine value results show that the content of unsaturated fatty acids decreases, the relative content of aldehydes produced by oxidation also decreases. In addition, enzymatic reactions may further reduce aldehydes by reducing the degree of peroxidation of fats and fats to reduce aldehydes to alcohols.



Note: Marking different letters indicates significant differences (P<0.05)
 Figure 10. Histogram of the content of volatile compounds in some beef tallow

2.5.3. Shea beef tallow volatile compound Venn diagram analysis.

As can be seen from Figure 11, the unrefined beef tallow contains 28 unique volatile substances, the refined beef tallow contains 25 unique volatile substances, and the beef tallow after adding enzymes contains 18 unique volatile substances, and there are 14 kinds of common volatile substances, namely trimethylbenzene, 2-methyldecane, n-dodecane, n-hexanol, trans-2-hexenal, 2-heptenal, n-heptanal, E-2-octenal, n-nonanal, trans-2-decelenal, 2-undecylenal, methylheptenone, palmitic acid, stearic acid, These compounds may play an important role in the aromatic qualities of beef tallow[14]. Each beef tallow had fewer volatile compounds and more unique volatile compounds, indicating that they were quite different, and all of them had their own unique characteristic compound combinations.

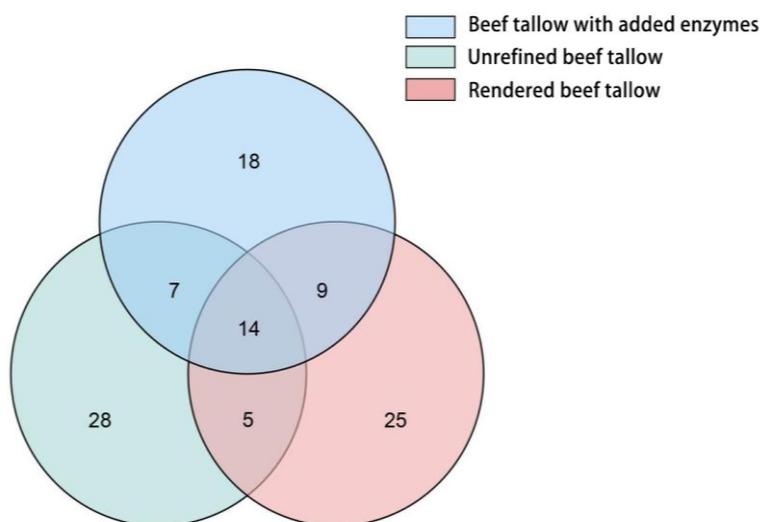


Figure 11. Wayne diagram of the volatile components of beef tallow

2.5.4. Principal component analysis of volatile flavor components of beef tallow.

Referring to the method of HJ Kim et al.[15] selecting volatile substances with relative content higher than 1% among six species of rhododendron for principal component analysis. In this study, the principal component analysis was carried out on 23 volatile components of unrefined beef tallow, refined beef tallow and beef tallow with a relative content of more than 1% after adding enzymes, and the obtained principal component score is shown in Fig. 12, the obtained principal component load is shown in Fig. 13, the eigenvalue and cumulative contribution rate of the correlation matrix are shown in Table 6, and the principal component load matrix and eigenvector are shown in Table 7. In order to more intuitively reflect the proportion of each compound in each principal component, each point on the three-dimensional load map was projected onto a plane to obtain a two-dimensional load map (Fig. 13).

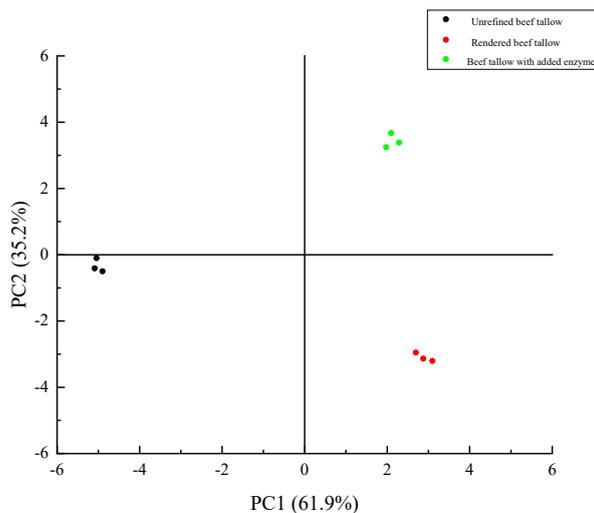


Figure 12. Principal component score

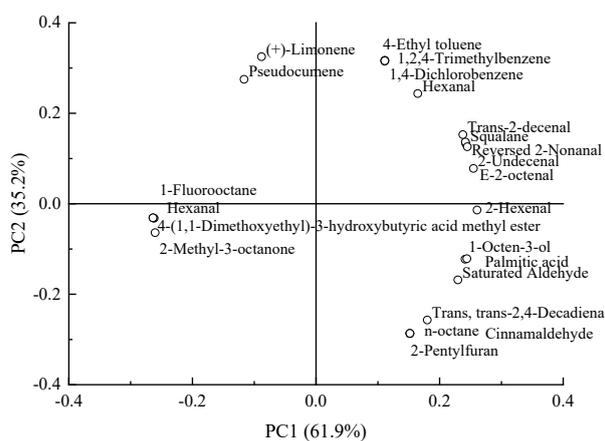


Figure 13. Principal component two-dimensional load diagram

Table 6. Eigenvalues and cumulative contribution rates of correlation matrices

Principal component	eigenvalue	Contribution rate/ %	Cumulative contribution rate / %
1	14.25	61.94	61.94
2	8.09	35.17	97.11

Table 7. Principal component load matrices and eigenvectors

Volatile components	Principal component 1		Principal component 2	
	Payload	Feature vector	Payload	Feature vector
n-octane X1	0.13	0.50	-0.30	0.87
Pseudocumene X2	0.03	0.13	0.34	0.99
4-Ethyl toluene X3	0.13	0.51	0.30	0.86
1,2,4-Trimethylbenzene X4	0.13	0.51	0.30	0.86
(+)-Limonene X5	-0.04	-0.14	0.34	0.99
Squalane X6	0.24	0.93	0.13	0.36
1-Octen-3-ol X7	0.25	0.96	-0.10	-0.29
Hexanal X8	-0.26	-1	0.002	0.006
2-Hexenal X9	0.25	0.98	-0.07	-0.2
Saturated Aldehyde X10	0.23	0.88	-0.17	-0.48
E-2-octenal X11	0.26	0.99	0.06	0.17
Cinnamaldehyde X12	0.129	0.50	-0.30	-0.87
Reversed 2-Nonanal X13	0.25	0.98	0.07	0.21
Hexanal X14	0.22	0.86	0.18	0.52
Trans, trans-2,4-Decadienal X15	0.16	0.60	-0.28	-0.80
Trans-2-decenal X16	0.25	0.96	0.10	0.28
2-Undecenal X17	0.25	0.96	0.10	0.29
2-Methyl-3-octanone X18	-0.26	-1	-0.03	-0.09
Palmitic acid X19	0.23	0.89	-0.16	-0.45
4-(1,1-Dimethoxyethyl)-3-hydroxybutyric acid methyl ester X20	-0.26	-1	0.002	0.006
1,4-Dichlorobenzene X21	0.13	0.51	0.30	0.86
1-Fluorooctane X22	-0.26	-1	0.002	0.006
2-Pentylfuran X23	0.129	0.50	-0.30	-0.87

According to the data in Tables 6 and 7, the cumulative contribution rate of the first and second principal components is 97.11%, which can fully cover most of the information contained in the original 23 variables. The contribution rate of the first principal component accounted for 61.94% of the total information, and the main reactions were n-heptanol, 1-octen-3-ol, 2-heptenal, E-2-octenal, trans-2-nonanal, trans-2-decenenal, and 2-undecylenal, and were negatively correlated with hexanal, 2-methyl-3-octanone, 4-(1,1-dimethylethoxy)-3-hydroxy-butyrates, and 1-fluorooctane, and negatively correlated with n-heptanol, 1-octen-3-ol, 2-heptenal, E-2-octenal, trans-2-nonanal, trans-2-decenal and 2-undecylenal were positively correlated. Table 6 shows that among the volatile components positively correlated with the first principal component, the beef tallow content after enzymatic hydrolysis reaction was high, and the main flavor was green aroma, fatty flavor, citrus flavor and oil aroma, and the flavor was good. Combined with the first principal component, the contribution rate was the largest, and from the perspective of flavor, it was preliminarily shown that lipase decomposition of beef tallow greatly improved the flavor of beef tallow. The contribution rate of the second principal component accounted

for 35.17% of the total information, which mainly reflected the information of n-octane, trimethylbenzene, (+)-limonene, 4-ethyltoluene, 1,2,4-trimethylbenzene and 2-pentylfuran, and was negatively correlated with n-octane, 2-pentylfuran and n-octanal, and positively correlated with trimethylbenzene, (+)-limonene, 4-ethyltoluene and 1,2,4-trimethylbenzene. Alkanes account for a high proportion of the second principal component, mainly because their relative content is high and the actual aroma effect is small, so the 2-pentylfuran and n-octal components contribute more to the second principal component, but these volatile components are negatively correlated with the flavor[14], and combined with the content of various volatile components, it can be preliminarily concluded that these negatively correlated components contribute greatly to the flavor of beef tallow after refining.

The cumulative contribution rate of the first two principal components reached 97.11%, and referencing the scoring method of soil characteristics processing by Gao Li et al.[16] for different treatment groups, establish the linear regression equations for the first two principal components PC1 and PC2. Use the variance contribution rates of the first and second principal components as the weight coefficients α_1 and α_2 to create a comprehensive evaluation model $S = \alpha_1 \times PC1 + \alpha_2 \times PC2$ to calculate the comprehensive score Y for beef tallow.

Table 8. Comprehensive scores and rankings of the three types of beef tallow

category	Y Overall score/ score	ranking / place
Unrefined beef tallow	-10.54	3
beef tallow after refining	2.41	2
beef tallow after adding enzymes	8.22	1

As can be seen from Table 8, Figure 12 and 13, the comprehensive score of beef tallow after adding enzymes is the highest, located in the first quadrant on the right side of Figure 12, 2-undecylenal, n-nonanal, 4-ethyltoluene, benzyl oleate, trans-2-nonanal, trans-2-decereal, methylheptenone, n-heptanol, n-octanol and so on are the most relevant volatile components, among which, trans-2-decaenal has a strong fragrance odor, 2-undecylenal has a fresh aldehyde odor, n-nonanal has a greasy odor and sweet orange odor. Therefore, from the perspective of volatile flavor analysis, the enzymatic hydrolysis reaction between lipase and beef tallow can improve the overall flavor of beef tallow.

3. Conclusion

Using beef tallow as raw material, the optimal combination of single factor experiment and orthogonal experiment was as follows: temperature 37 °C, time 80 min, and enzyme dosage of 0.04 mg/g. Under these conditions, the highest sensory score is 8.94, and the BP algorithm is used to predict the senses, and the fitting degree is 0.978, which means that the model has certain rationality and practical application value. The results showed that aldehydes contributed significantly to the flavor of beef tallow, and the contents of aldehydes and ketones in avocado were significantly decreased ($P < 0.05$), and alcohols were significantly increased ($P < 0.05$), and the highest comprehensive score was 8.22, which was significantly higher than that of other groups. This indicated that the enzymatic hydrolysis reaction effectively improved the flavor of beef tallow and made it reach the best flavor quality, and the study of Xiang et al. [17] also proved that enzymatic hydrolysis can significantly enhance the overall aroma intensity of tallow. The flavor suitability evaluation method established in this study can identify the effect of lipase on beef tallow flavor, provide a theoretical basis for beef tallow flavor control, and also provide a reference for flavor optimization of other oil products, which has important theoretical significance and practical application value.

Acknowledgments

This work was financially supported by Key R&D Program of Shandong Province, China , 2023TZXD046; Research and Development and Demonstration of Key Technologies for Processing and Application of Prefabricated Compound Condiments, Shandong Province Technology Innovation Leading Plan (Central Government's Guiding Funds for Local Scientific and Technological Development) , YDZX2023088; sgyhx2023-56; Key R&D Program of ZiBo City, China,2021SNPT0007 fund.

References

- [1] Y. Wang, K. Wang, X. Xiang, S. Zhang, J. Huang, C. Tang, Q. Wang, A novel complex of pectin-crosslinked β -cyclodextrin: Effectively reducing cholesterol content in hotpot beef tallow, *J Food Sci* 90 (2025). <https://doi.org/10.1111/1750-3841.70056>.
- [2] M. Ghasemi-Varnamkhashti, S.S. Mohtasebi, M.L. Rodriguez-Mendez, J. Lozano, S.H. Razavi, H. Ahmadi, C. Apetrei, Classification of non-alcoholic beer based on aftertaste sensory evaluation by chemometric tools, *Expert Syst Appl* 39 (2012) 4315–4327. <https://doi.org/10.1016/j.eswa.2011.09.101>.
- [3] Q. DONG, BP NEURAL NETWORK FOR EVALUATING SENSORY TEXTURE PROPERTIES OF COOKED SAUSAGE, *J Sens Stud* 24 (2009) 833–850. <https://doi.org/10.1111/j.1745-459X.2009.00240.x>.
- [4] A. Qi, Research on Prediction Model of Improved BP Neural Network Optimized by Genetic Algorithm, in: *Proceedings of the 2017 4th International Conference on Machinery, Materials and Computer (MACMC 2017)*, Atlantis Press, Paris, France, 2018. <https://doi.org/10.2991/macmc-17.2018.145>.
- [5] Application of BP neural network model based on whale algorithm optimization in text word separation, *Academic Journal of Mathematical Sciences* 4 (2023). <https://doi.org/10.25236/AJMS.2023.040404>.
- [6] H. Pan, J. Yang, Y. Shi, T. Li, BP Neural Network Application Model of Predicting the Apple Hardness, *J Comput Theor Nanosci* 12 (2015) 2802–2807. <https://doi.org/10.1166/jctn.2015.4180>.
- [7] R. Chen, N. Lin, J. Su, Y. Shi, BP Neural Network-based Model for Evaluating User Interfaces of Human-computer Interaction System, in: *Proceedings of the 3rd International Conference on Mechatronics Engineering and Information Technology (ICMEIT 2019)*, Atlantis Press, Paris, France, 2019. <https://doi.org/10.2991/icmeit-19.2019.112>.
- [8] A. Sharma, P.K. Rai, S. Prasad, GC–MS detection and determination of major volatile compounds in Brassica juncea L. leaves and seeds, *Microchemical Journal* 138 (2018) 488–493. <https://doi.org/10.1016/j.microc.2018.01.015>.
- [9] S. Wu, L. Shen, Y. Li, L. Qin, X. Yang, Dynamic changes in gut microbiota during the intervention of tea polyphenols to alleviate high-fat diet-induced obesity, *Food Science and Human Wellness* (2025). <https://doi.org/10.26599/FSHW.2025.9250502>.
- [10] A. Qi, Research on Prediction Model of Improved BP Neural Network Optimized by Genetic Algorithm, in: *Proceedings of the 2017 4th International Conference on Machinery, Materials and Computer (MACMC 2017)*, Atlantis Press, Paris, France, 2018. <https://doi.org/10.2991/macmc-17.2018.145>.
- [11] K. Guo, Q. Wang, T. Xia, L. Wang, H. Song, L. Yang, Effect of smelting temperatures on the odor compounds of beef tallow through instrumental and sensory techniques, *Journal of Food Composition and Analysis* 119 (2023) 105280. <https://doi.org/10.1016/j.jfca.2023.105280>.
- [12] S. Song, Q. Tang, L. Fan, X. Xu, Z. Song, K. Hayat, T. Feng, Y. Wang, Identification of pork flavour precursors from enzyme-treated lard using Maillard model system assessed by GC–MS and partial least squares regression, *Meat Sci* 124 (2017) 15–24. <https://doi.org/10.1016/j.meatsci.2016.10.009>.
- [13] Q. Liu, J. Lin, W. Zhao, M. Lei, J. Yang, W. Bai, The dynamic changes of flavors and UPLC-Q-Exactive-Orbitrap-MS based lipidomics in mackerel (*Scomberomorus niphonius*) during dry-cured

- processing, *Food Research International* 163 (2023) 112273. <https://doi.org/10.1016/j.foodres.2022.112273>.
- [14] S. Tong, W. Li, Y. Rao, Y. Xiao, Y. Yan, W. Guo, X. Lü, J. Sun, L. Ai, L. Ni, Microbiomics and metabolomics insights into the microbial regulation on the formation of flavor components in the traditional fermentation process of Chinese Hongqu aged vinegar, *Food Science and Human Wellness* 13 (2024) 2765–2778. <https://doi.org/10.26599/FSHW.2022.9250224>.
- [15] H.-J. Kim, D.-L. Hong, J.-W. Yu, S.-M. Lee, Y.-B. Lee, Identification of Headspace Volatile Compounds of Blended Coffee and Application to Principal Component Analysis, *Prev Nutr Food Sci* 24 (2019) 217–223. <https://doi.org/10.3746/pnf.2019.24.2.217>.
- [16] L. Gao, R. Li, D. Yang, L. Bao, N. Zhang, Phosphogypsum improves soil and benefits crop growth: An effective measure for utilizing solid waste resources, *Sci Rep* 15 (2025) 11827. <https://doi.org/10.1038/s41598-025-97216-8>.
- [17] X. Xiang, K. Wang, F. Wang, Q. Yang, J. Huang, Q. Zhou, Q. Wang, Enhancing beef tallow flavor through enzymatic hydrolysis: Unveiling key aroma precursors and volatile compounds using machine learning, *Food Chem* 477 (2025) 143559. <https://doi.org/10.1016/j.foodchem.2025.143559>.