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Optimize the Process and Quality Analysis of Flavored Cowhide Based on the Fuzzy Mathematics Sensory Evaluation Method

Chunxia Chu, Guifang Chang, He Zhu *

School of Food Science and Engineering, Shandong Agriculture and Engineering University, Jinan, China

ABSTRACT

Flavored cowhide was made using fresh cowhide as raw materials. Based on the single-factor experiment, through fuzzy mathematical sensory evaluation combined with the response surface experiment, with color, taste, hardness, elasticity and toughness, and tissue state as sensory indicators, the process of flavored cowhide was optimized. The experimental results show that the optimal process parameters are: The ratio of material to liquid is 0.70 g/ml, the boiling time is 60 min, and the soaking time is 45 h. The effect is the best at this time, with a sensory score of 90.36 points. On this basis, the changes in its physical properties, microstructure and molecular structure were determined by using a texture analyzer, SEM scanning electron microscope and Fourier transform infrared spectrometer. The research of this experiment is expected to provide certain references for the application of cowhide in the field of food processing.

KEYWORDS

Cowhide; Sensory evaluation of fuzzy mathematics; Process optimization; Response surface method

1. INTRODUCTION

The Shi Yi Xin Jian records that cowhide possesses the effects of "promoting diuresis to reduce edema and detoxifying," endowing it with certain medicinal value [1]. Beyond this, cowhide also exhibits high nutritional value: its collagen content accounts for 26% of the total protein in fresh cowhide—approximately 80% of the total protein content—while its fat content constitutes about 8%, classifying it as a high-protein, low-fat animal-derived food [2]. Collagen plays a pivotal role in the unique physiological functions of bone, skin, tendons, and cartilage tissues [3], and is beneficial for muscle growth and repair, as well as for maintaining skin elasticity and radiance [4]. Additionally, cowhide is rich in various nutrients, including vitamin B, vitamin E, calcium, magnesium, potassium, sodium, and selenium, which help enhance immune function, stimulate metabolism, and support nervous system integrity [5]. With rising living standards, there has been a growing demand for healthy diets and aesthetic appeal, leading to widespread interest in low-fat foods rich in collagen. China possesses one of the most distinctive agro-pastoral transition zones in the world [6]. According to survey data, China is a major producer and consumer of beef cattle, having consistently maintained its position as the world's largest importer of beef over the past several decades [7]. However, current processing of cowhide in the market is largely confined to leather products, and the utilization rate of tannery processes remains low, resulting in soil and water pollution that compromises environmental hygiene [8, 9]. Food-based applications of cowhide remain limited; in Longyan City, Fujian Province, there exists a small-scale artisanal tradition of preparing ready-to-eat soaked cowhide, yet such

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artisanal production relies heavily on empirical knowledge, making it difficult to precisely control processing parameters—a factor that compromises flavor consistency and product quality stability [9]. Therefore, by employing a scientific and systematic experimental protocol that integrates traditional culinary practices with modern food processing technologies, this study aims to overcome the technical challenges associated with the industrial production of cowhide-based food products. By conducting in-depth investigations into the effects of ingredient-to-liquid ratio, boiling temperature, and soaking duration on product quality, and by leveraging fuzzy mathematical sensory scoring to establish a precise evaluation framework, this approach not only enables standardized and quantified control of process parameters—thereby ensuring consistent flavor and quality of the final product—but also utilizes response surface methodology to optimize the optimal process combination. Such an initiative can transform cowhide from a single raw material for leather processing into a diversified, high-value food product line; reduce environmental pollution associated with the tanning industry; and potentially provide consumers with novel snack foods that combine health benefits with distinctive flavor profiles. This strategy will thus facilitate innovative development pathways for the food industry and promote the efficient, integrated utilization of agricultural and livestock resources.

2. MATERIALS AND METHODS

2.1. Materials and Equipment

2.1.1. Experimental Materials

Cowhide, sourced from the ecological mountain pastures in Nanning, Guangxi Province. Chili pepper essential oil, provided by Qingdao Weixianglin Food Additives Co., Ltd.; yeast extract, supplied by Henan Gaobao Industrial Co., Ltd.; sodium glutamate, supplied by Lianhua Health Industry Food Co., Ltd.; disodium flavor nucleotides, supplied by Shenzhen Xingmu Bioengineering Co., Ltd.; table salt, supplied by China Salt Dongxing Yunmeng Salt.

2.1.2. Instruments and Equipment

Electronic analytical balance, Metrohm Instruments (Shanghai) Co., Ltd.; vacuum freeze dryer, Shandong Sanbi Instrument Co., Ltd.; Zeiss field-emission transmission electron microscope, SQI Technology (Beijing) Co., Ltd.; texture analyzer, Food Technology Corporation (USA); forced-air drying oven, Gongyi Yuhua Instrument Co., Ltd.; Fourier transform infrared spectrometer, Thermo Fisher Scientific IS5.

2.2. Experimental Methods

2.2.1. Experimental Processes

Following the method for preparing ready-to-eat marinated beef skin described by Li Ying et al. [9], minor modifications were made. The preparation of flavored beef skin follows the procedure outlined below. The key steps are as follows: the beef skin is thawed, thoroughly cleaned, and then boiled in water for 60 min using an electromagnetic cooker set to 2200 V; after cooking, the surface fat is removed, and the meat is cut into strips measuring 0.8 cm × 5 cm while still hot; the strips are then placed in cold water to prevent them from adhering to each other; subsequently, 40 g of beef skin is immersed in the prepared soaking solution (containing 0.6 g salt, 1 g sugar, 0.1 g sodium glutamate, 0.005 g disodium inosinic acid, 8 g chili oil, 0.175 g yeast extract, and 24 g water) and soaked in a refrigerator for 45 h; finally, the soaked beef skin is removed, vacuum-packaged, and stored under low-temperature refrigeration.

Calf skin → Thawing → Cleaning → Boiling → Trimming → Slicing → Cooling → Weighing → Preparing the soaking solution → Soaking → Vacuum packaging → Finished product → Cold storage

2.2.2. Single-factor Experimental Design

This study employed fuzzy mathematics and comprehensive sensory scoring to investigate the sensory characteristics of leather obtained under varying material-to-liquid ratios, boiling durations, and soaking durations, thereby optimizing the parameter levels for each factor. In this experiment, while keeping all other conditions constant, single-factor experiments were conducted for each of the three variables—material-to-liquid ratio, boiling duration, and soaking duration—to preliminarily determine the optimal ranges for these parameters in leather production. Forty grams of fresh cowhide were placed in boiling water and boiled for 50 min, 55 min, 60 min, 65 min, and 70 min, respectively; after boiling, the hides were removed and placed in cold water. The boiled hides were then mixed with soaking solutions prepared at material-to-liquid ratios of 0.56 g/ml, 0.64 g/ml, 0.72 g/ml, 0.80 g/ml, and 0.86 g/ml, followed by soaking for 12 h, 24 h, 48 h, 60 h, and 72 h, respectively; all other ingredients were weighed according to standard specifications. By examining the effects of varying material-to-liquid ratios, boiling durations, and soaking durations on the sensory quality of the leather, this study utilized sensory evaluation to identify the optimal parameters for each factor.

2.2.3. Response Surface Methodology

Building upon a single-factor experiment, this study employed fuzzy mathematical sensory scoring as the response variable (Y) and designed a three-factor, three-level response surface optimization experiment involving three factors—feed-to-liquid ratio (A), boiling time (B), and soaking time (C)—to optimize the parameters of flavored cowhide using response surface methodology. The experimental factor and level design is presented in Table 1.

Table 1. Response Surface Factors and Levels

Levels	A Feed-to-liquid ratio/(g/mL)	B Cooking time/min	C Soak period/h
-1	0.64	55	24
0	0.72	60	48
1	0.80	65	72

2.2.4. Establishing a Fuzzy Mathematical Sensory Evaluation Model

Following the methodology proposed by Yu Jianxia et al. [10], a fuzzy mathematical sensory evaluation model was established.

(1) Organoleptic Investigation

A panel of evaluators—consisting of 10 participants (5 males and 5 females) who had undergone sensory evaluation training—was selected to conduct sensory evaluations of flavored cowhide. The evaluations encompassed the color, flavor, firmness, elasticity, and texture of the flavored cowhide; based on product quality differences, the samples were classified into four grades: excellent, good, medium, and poor. The sensory evaluation criteria [9] are presented in Table.

Table 2. Sensory Evaluation Criteria Table

grade	colour and lustre	taste	hardness	Elasticity	Organizational status
V1 (90–100 points)	Has a lustrous appearance with uniform distribution	Subtle spicy flavor with a rich mouthfeel	Crisp texture with excellent chewiness	firm texture with excellent chewability	The organizational structure is compact and evenly distributed.
V2 (80–90 points)	The color is translucent, and the distribution is relatively uniform.	Slightly spicy flavor with a balanced taste profile.	Balanced in texture, with moderate chewiness.	The texture is relatively firm, with a moderate chewiness.	The organizational structure is relatively evenly distributed.
V3 (60–80 points)	The color is dull and slightly uneven.	There is an off odor, and the flavor is abnormal.	It has a soft, chewy texture but lacks a firm bite.	Poor texture compactness, but high masticatory force	Uneven tissue distribution
V4 (0–60 points)	The color is dark yellow, and the distribution is uneven.	Lacks aroma, with a bland flavor profile.	Insufficient hardness and poor mouthfeel	Loose texture, with insufficient elasticity and toughness	Inconsistent quality and poor chewability

(2) Define the Set of Evaluation Criteria And The Set of Evaluation Levels

Let the set of evaluation criteria for flavored beef products be denoted by U, and the set of grade levels be denoted by V.

$$U = |\text{Color (U1)} \quad \text{Taste (U2)} \quad \text{Hardness (U3)} \quad \text{Elasticity (U4)} \quad \text{Tissue state (U4)}|$$

$$V = |\text{Excellent (V1)} \quad \text{Good (V2)} \quad \text{Medium (V3)} \quad \text{Poor (V4)}|$$

(3) Determine the Weight Set

A questionnaire survey was conducted among 100 participants, consisting of faculty members or students specializing in food science. The weight set for the sensory evaluation was denoted as X.

$$X = |\text{Color (X1)} \quad \text{Taste (X2)} \quad \text{Hardness (X3)} \quad \text{Elasticity (X4)} \quad \text{Tissue state (X5)}|$$

Based on the proportional analysis, the weighting table is presented in Table 3.

Table 3. Weights of Individual Factors for Flavored Cattle Leather

factor	colour and lustre	taste	hardness	Elasticity	Organizational status
weighted valueX/%	20	25	20	25	10

(4) Construct the Fuzzy Matrix

Ten evaluators were invited to assess five evaluation criteria for each sample; the number of votes obtained by each sample for each criterion was then divided by the total number of evaluators (10) to derive the respective fuzzy matrices T.

(5) Fuzzy Mathematical Comprehensive Evaluation Set

Let Y denote the fuzzy comprehensive sensory evaluation set, where $Y = X \cdot T$. Here, X represents the sensory evaluation weight set, and T denotes the fuzzy matrix.

(6) Fuzzy Mathematical Sensory Scoring

Let G denote the fuzzy mathematical sensory scoring set, where $G = Y \cdot V$. Here, Y represents the fuzzy mathematical comprehensive evaluation set, and V denotes the set of evaluation levels.

2.2.5. Microstructural Characterization By Scanning Electron Microscopy

Cut samples (8 mm×5 mm×2 mm) were immersed in a series of ethanol solutions with progressively increasing concentrations (30%,50%,70%,80%,90%,95%,100%) for 10–15 minutes each, to gradually remove moisture from the samples. After being transferred to a −80 °C freezer, the samples were removed and subjected to drying using a vacuum freeze dryer.

2.2.6. Texture Determination

Measurements were performed using a TA.XT Plus texture analyzer. Three types of samples—fresh raw cowhide, cooked but unprocessed cowhide, and flavored cowhide—were each cut into cubes measuring 2 cm × 2 cm × 1 cm. Following the methodologies described by Ma Xiangjie [11] and Sun Cailing [12], with minor modifications, the following parameters were set: probe type P/0.5; pre-test speed of 2 mm/s; test and post-test speeds of 1 mm/s; trigger type Auto (Force) 5 g; downforce distance of 5 mm; and probe testing speed of 2.5 mm/s. Each sample was tested in triplicate, and the average values were calculated. The texture parameters measured were hardness, adhesiveness, springiness, cohesiveness, elasticity, stickiness, and chewiness.

2.2.7. Determination by Fourier Transform Infrared Spectroscopy

The NICOLET 6700 infrared spectrometer was employed, with a spectral recording range of 400–4000 cm^{-1}, 16 scans, and a resolution of 4 cm^{-1}; all tests were conducted under dry conditions at 25 ± 1 °C. Each experiment was repeated three times, and the average spectrum was calculated for further analysis.

2.2.8. Date Analysis

Statistical analyses were performed using Excel, SPSS 16, Design-Expert 13, and OMNIC software, while graphical representations were created using Origin 2021 software.

3. RESULTS AND ANALYSIS

3.1. Experiment of Single Factor

3.1.1. Effect of Feed Solution Ratio on the Sensory Evaluation of Cowhide

As shown in Figure 1, when the material-to-liquid ratio increases from 0.56 g/ml to 0.72 g/ml, the sensory score exhibits an upward trend; however, when the ratio continues to increase from 0.72 g/ml to 0.88 g/ml, the sensory score shows a downward trend. Overall, the data follow an inverted "V" - shaped pattern—initially rising and then declining. At a material-to-liquid ratio of 0.56 g/ml, the sensory score was only 78.42, significantly lower than that at 0.72 g/ml. This may be attributed to an excessively low ratio, i.e., an excess of water. Excessive water dilutes the concentration of flavor compounds, thereby weakening the flavor profile of the leather. Moreover, when too much water is

absorbed by the leather, it may result in excessive softness, reduced elasticity and toughness, and a loose texture; these combined effects lead to suboptimal sensory scores. At a material-to-liquid ratio of 0.72 g/ml, the sensory score reaches its peak value of 90.09. At this ratio, the proportion of water to raw materials is optimal. An appropriate amount of water facilitates the full extraction of flavor compounds and their uniform distribution throughout the leather, yielding an appealing color and a rich flavor. Simultaneously, it ensures that the leather possesses adequate hardness, elasticity, and toughness, along with a uniform and compact texture. This balance across all sensory indicators results in the highest sensory score. At a material-to-liquid ratio of 0.86 g/ml, the sensory score drops to its lowest value of 78.38. In this scenario, an excessively high ratio leads to an overly concentrated product, which can induce off-flavors. Furthermore, the salt content in the raw materials may not dissolve or disperse adequately, resulting in suboptimal color and flavor, as well as a texture that is too hard and lacks sufficient elasticity and toughness—all of which contribute to a lower sensory score. Therefore, the appropriate material-to-liquid ratio should be selected within the range of 0.64–0.8 g/ml.

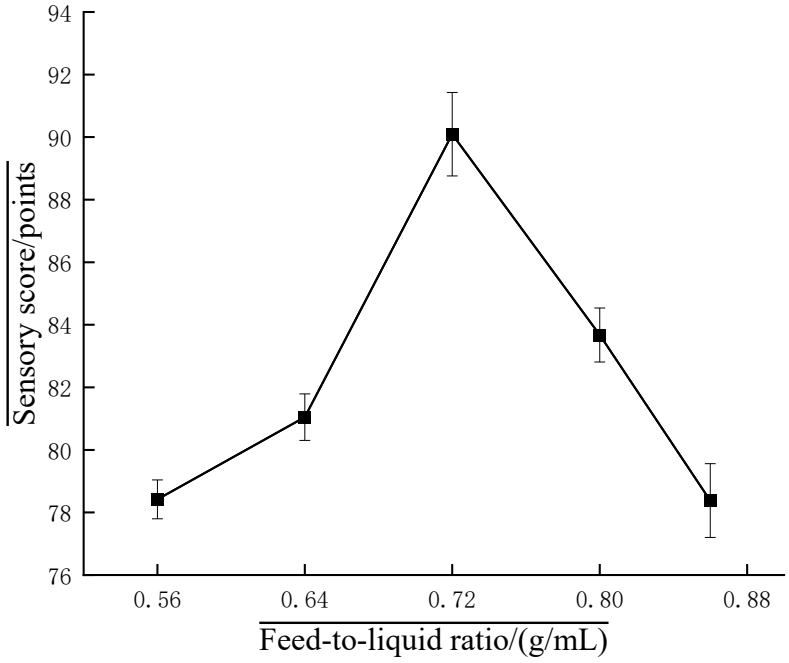


Figure 1. Effect of feed-to-liquid ratio on sensory scoring of cowhide

3.1.2. Effect of Cooking Time on the Sensory Evaluation of Cowhide

As shown in Figure 2, when the boiling duration increased from 50 min to 60 min, the sensory score exhibited an upward trend; however, when the boiling duration was further increased from 60 min to 70 min, the sensory score showed a downward trend. The overall pattern followed an inverted "V" - shaped curve—initially rising and then declining. At a boiling duration of 50 min, the average sensory score was 77.25, significantly lower than that at 60 min. This may be attributed to insufficient boiling time, which resulted in incomplete cooking of the cowhide, leading to a denser internal structure, higher hardness, and inferior mouthfeel. Concurrently, flavor compounds could not fully penetrate the cowhide, resulting in less rich flavor, less ideal coloration, and a less uniform texture—all of which collectively contributed to the lower sensory score. At 60 min, the sensory score reached its peak value of 88.92. At this optimal boiling duration, the internal structure of the cowhide underwent complete transformation, achieving an optimal balance between hardness and elasticity, and yielding an appropriate mouthfeel. Furthermore, flavor compounds were fully penetrated and integrated, resulting in a richer flavor profile, superior coloration, and a uniform, fine texture. All sensory indicators thus reached optimal levels, accounting for the highest sensory score. At 70 min, the average sensory score decreased to 76.11—the lowest value observed. This decline may be due to excessive boiling time, which caused the cowhide to become overly soft and lose its inherent elasticity

and resilience. Additionally, prolonged boiling could lead to the volatilization or chemical alteration of certain flavor compounds, further degrading the flavor profile and resulting in suboptimal sensory scores. Taking all factors into account, the optimal cooking time is 55–65 minutes.

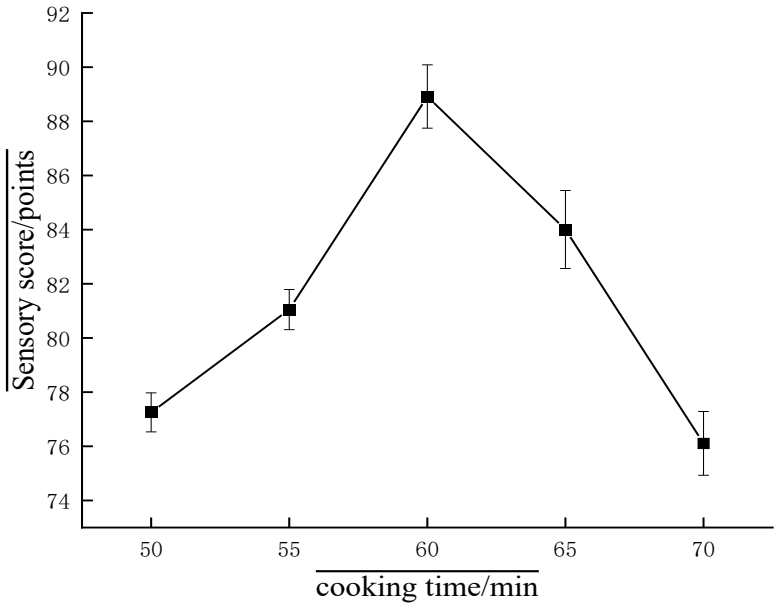


Figure 2. Effect of cooking time on the sensory scores of cowhide

3.1.3. Effect of Soaking Duration on the Sensory Evaluation of Leather Quality

As shown in Figure 3, the sensory scores of cowhide gradually increased as the soaking duration extended from 24 h to 48 h; however, when the soaking duration was further increased from 48 h to 72 h, the sensory scores exhibited a declining trend. The overall pattern followed a parabolic curve—initially rising and then decreasing. At a soaking duration of 24 h, the average sensory score was 87.65, which was relatively low. This may be attributed to the fact that, with a shorter soaking period, the cowhide could not fully absorb the diverse flavor compounds present in the soaking solution, resulting in a flavor profile that lacked richness and fullness, as well as slightly inferior mouthfeel and aroma, thereby adversely affecting the overall sensory score. At a soaking duration of 48 h, the sensory score reached its peak value of 89.23. This duration proved to be optimal: the cowhide had sufficient time to absorb the flavor components from the soaking solution, yielding a rich flavor profile, harmonious mouthfeel, and favorable coloration and texture. The various sensory indicators complemented each other, achieving an optimal balance, which consequently led to the highest sensory score. At a soaking duration of 72 h, the average sensory score decreased to 87.38. This decline can be attributed to prolonged soaking potentially causing the cowhide to absorb excessive moisture, leading to softening of the texture and reduced elasticity and toughness. Simultaneously, flavor compounds may have permeated excessively or undergone chemical changes, resulting in flavor imbalance and undesirable mouthfeel, which in turn led to suboptimal sensory scores. In summary, the recommended soaking duration is 36–60 h.

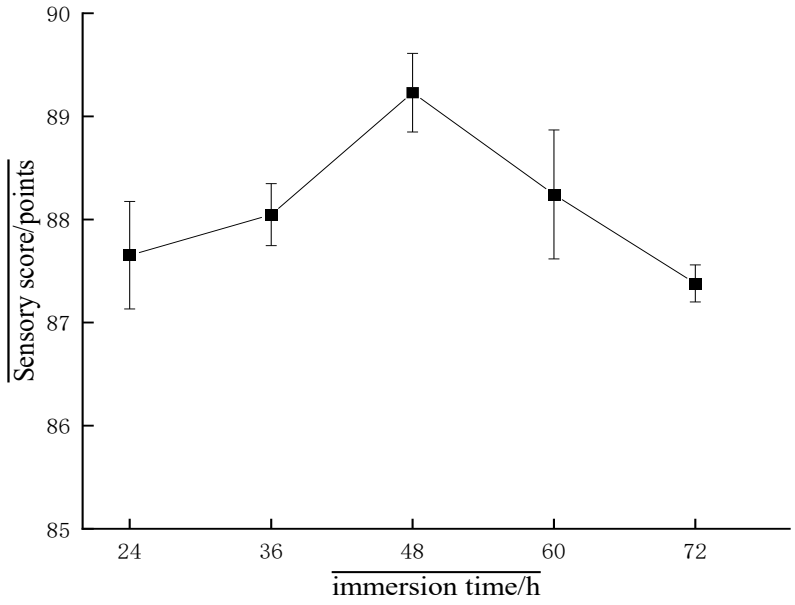


Figure 3. Effect of immersion time on the sensory scores of cowhide

3.2. Results of Fuzzy Mathematical Sensory Evaluation and Construction of the Fuzzy Matrix

Ten individuals with expertise in food science were selected to score five evaluation criteria for 17 batches of flavored beef samples; the results are presented in Table 4.

Table 4. Statistical Summary of Sensory Evaluation Indicators for Flavored Cattle Leather

sample	colour and lustre				taste				hardness				Elasticity				Organizational status			
	v1	v2	v3	v4	v1	v2	v3	v4	v1	v2	v3	v4	v1	v2	v3	v4	v1	v2	v3	v4
1	6	1	3	0	4	4	2	0	5	3	2	0	5	3	2	0	3	3	4	0
2	6	2	0	2	4	3	2	1	4	4	1	1	4	5	1	1	4	4	2	0
3	4	3	1	2	4	4	2	0	5	3	2	0	5	3	2	0	2	3	4	1
4	4	3	3	0	3	4	2	1	4	4	1	1	4	5	1	1	4	4	1	1
5	5	3	2	0	5	5	0	0	5	4	1	0	7	3	0	0	4	4	2	0
6	4	6	1	0	7	1	2	0	5	4	1	0	4	5	0	1	6	2	1	1
7	4	4	2	0	5	4	1	0	5	4	1	0	6	3	1	0	6	4	0	0
8	5	4	1	0	5	3	1	1	6	3	1	0	6	2	2	0	5	5	0	0
9	5	4	1	0	5	3	1	1	6	3	1	0	6	1	3	0	5	5	0	0
10	4	3	2	1	4	4	2	0	5	3	2	0	5	3	2	0	5	2	3	0
11	5	4	0	1	5	3	1	1	6	3	1	0	5	4	1	0	6	2	1	1
12	5	4	0	1	5	3	1	1	6	2	2	0	5	4	1	0	6	2	0	2
13	8	2	0	0	8	2	0	0	9	1	0	0	6	2	2	0	6	2	1	1
14	8	2	0	0	8	1	0	1	8	2	0	0	8	1	1	0	7	2	1	0
15	8	1	0	1	8	2	0	0	7	2	1	0	8	1	1	0	9	1	0	0
16	8	2	0	0	8	2	0	0	9	1	0	0	7	1	1	1	7	2	1	0
17	8	2	0	0	8	1	0	1	8	2	0	0	8	1	1	0	7	3	0	0

By referencing the distribution of ratings (excellent, good, medium$\frac{\text{excellent}-\text{rating vote count}}{\text{total number of panelists}}$, poor) among the evaluators for each experimental product, a fuzzy matrix ranging from 1 to 17 was constructed for each experimental product, with ratings assigned in descending order of quality. Through matrix multiplication, the synthesized sensory scores were calculated, thereby mitigating errors associated with the additive scoring method. For the flavor beef samples, the five weight sets

were defined as X={0.20,0.25,0.20,0.25,0.10}, and the evaluation set as V={95,85,70,30}. The evaluation results for the samples under varying experimental conditions were subsequently derived as follows:

Taking Sample 1 as an example, the fuzzy evaluation matrix is

$$T1=\begin{vmatrix}0.6 & 0.1 & 0.3 & 0\\ 0.4 & 0.2 & 0.2 & 0\\ 0.5 & 0.3 & 0.2 & 0\\ 0.5 & 0.3 & 0.2 & 0\\ 0.3 & 0.3 & 0.4 & 0\end{vmatrix}$$, The comprehensive evaluation matrix is as follows: $Y1 = X \times T1 =$

$$|0.20 \quad 0.25 \quad 0.20 \quad 0.25 \quad 0.10| \times \begin{vmatrix}0.6 & 0.1 & 0.3 & 0\\ 0.4 & 0.2 & 0.2 & 0\\ 0.5 & 0.3 & 0.2 & 0\\ 0.5 & 0.3 & 0.2 & 0\\ 0.3 & 0.3 & 0.4 & 0\end{vmatrix} = |0.485 \quad 0.285 \quad 0.240 \quad 0|$$, The same procedure is applied to Y_2- Y_{17}, yielding the comprehensive evaluation results presented in Table 5.

The fuzzy mathematical sensory score is calculated as $G1 = Y1 \times V1 = |0.485 \quad 0.285 \quad 0.240 \quad 0| \times \begin{vmatrix}95\\ 85\\ 70\\ 30\end{vmatrix} = 86.15$; similarly, the scores are obtained as G2 = 83.75, G3 = 83.50, G4 = 84.00, G5 = 88.40, G6 = 88.43, G7 = 88.50, G8 = 87.35, G9 = 86.98, G10 = 85.30, G11 = 86.08, G12 = 85.38, G13 = 91.05, G14 = 91.00, G15 = 91.13, G16 = 90.95, and G17 = 91.15.

Table 5. Comprehensive Evaluation Results

Yi	Evaluation result set
Y2	|0.44 0.6 0.115 0.11|
Y3	|0.425 0.325 0.2 0.05|
Y4	|0.375 0.405 0.165 0.08|
Y5	|0.54 0.38 0.08 0|
Y6	|0.515 0.37 0.1 0.035|
Y7	|0.515 0.375 0.11 0|
Y8	|0.545 0.315 0.115 0.025|
Y9	|0.545 0.29 0.14 0.025|
Y10	|0.455 0.315 0.21 0.02|
Y11	|0.53 0.335 0.08 0|
Y12	|0.53 0.315 0.09 0.065|
Y13	|0.75 0.18 0.06 0.01|
Y14	|0.44 0.6 0.115 0.11|
Y15	|0.425 0.325 0.2 0.05|
Y16	|0.375 0.405 0.165 0.08|
Y17	|0.54 0.38 0.08 0|

3.3. Response Surface Experimental Analysis and Results

3.3.1. Response Surface Experimental Design and Fuzzy Mathematical Sensory Evaluation Results

Using Design-Expert 13 software, multiple regression analysis was performed on the data in Table 6 to establish the regression model for the response variable Y in terms of variables A (material-to-liquid ratio), B (boiling time), and C (soaking time).

Code the regression equation:

$$Y=+91.06-0.3775A-0.5975B-0.2250C+0.7250AB-0.2950AC+0.2450BC-2.24A^2-4.47B^2-0.6505C^2$$

Actual value regression equation:

$$Y=-646.48600++396.89375A+19.93590B++0.087167C++1.81250AB-0.153646AC+0.002042BC-349.29687A^2-0.178820B^2-0.001129C^2$$

Table 6. Box-Behnken experimental design and results

Experiment No.	A: Material-to-liquid ratio / (g/ml)	B: Cooking time /min	C: soak period/h	response
1	0.72	60	48	91.15
2	0.72	60	48	90.95
3	0.72	60	48	91.13
4	0.72	60	48	91
5	0.72	60	48	91.05
6	0.72	65	72	85.38
7	0.72	55	72	86.08
8	0.72	65	24	85.3
9	0.72	55	24	86.98
10	0.8	60	72	87.35
11	0.64	60	72	88.5
12	0.8	60	24	88.43
13	0.64	60	24	88.4
14	0.8	65	48	84
15	0.64	65	48	83.5
16	0.8	55	48	83.75
17	0.64	55	48	86.15

3.3.2. ANOVA of the Response Surface Experimental Results

An analysis of variance (ANOVA) was conducted on the aforementioned regression equation, and the results are presented in Table 7. The F-value of the regression model was 874.47, with a P-value <0.0001, indicating highly significant differences; the lack-of-fit term was not significant ($P = 0.1201 > 0.05$). The R^2 and $RAdj^2$ values were 0.9991 and 0.9980, respectively, and the adjusted correlation coefficients for both terms were close to 1. Additionally, the coefficient of variation (CV) was 0.1418%, demonstrating that the predicted sensory scores for flavorized beef leather exhibited good agreement with the actual values, confirming that the established model had excellent goodness-of-fit. Based on the magnitude of the F-values, the order of significance of the factors influencing the fuzzy sensory score response values was as follows: cooking time (B)> feed-to-meat ratio (A)> soaking time (C). The quadratic terms B^2, A^2, and C^2 were all highly significant (P <0.0001). Regarding the interaction effects between the two factors, only AB showed highly significant differences, AC showed significant differences, while BC showed no significant difference.

Table 7. ANOVA Table for the Regression Model

Variance sources	quadratic sum	Degrees of freedom	Mean square	Fprice	Pprice	conspicuousness
model	121.45	9	13.49	874.47	< 0.0001	significant
A	1.14	1	1.14	73.88	< 0.0001	
B	2.86	1	2.86	185.08	< 0.0001	
C	0.4050	1	0.4050	26.25	0.0014	
AB	2.10	1	2.10	136.25	< 0.0001	
AC	0.3481	1	0.3481	22.56	0.0021	
BC	0.2401	1	0.2401	15.56	0.0056	
A^2	21.04	1	21.04	1363.58	< 0.0001	
B^2	84.15	1	84.15	5453.09	< 0.0001	
C^2	1.78	1	1.78	115.46	< 0.0001	
residual	0.1080	7	0.0154			
Unspecified item	0.0793	3	0.0264	3.68	0.1201	not significant
Error term	0.0287	4	0.0072			
amount to	121.56	16				

3.3.3. Analysis of the Two-Factor Interaction in the Response Surface Methodology Experiment

Using the equations derived from each experimental run, we employed Design-Expert software to generate three-dimensional response surface plots and contour maps illustrating the interactive effects of three factors on the response variable. By analyzing the topology of the mesh in the three-dimensional surface plots in conjunction with the density of the contour lines in the contour maps, we can infer the interrelationships among the three influencing factors. In the response surface plot, a steeper slope indicates that the interaction among these factors exerts a greater influence on the fuzzy sensory scores. In the contour map, an elliptical contour signifies a strong interaction between two factors, whereas a circular contour indicates a weak interaction.

(1) Comparison of the Significance of the Interaction Between The Material-To-Liquid Ratio And Cooking Time on the Fuzzy Sensory Scores

By analyzing the interactive response surface plot of material-to-liquid ratio A and cooking time B in Figure 4, it can be observed that, when the material-to-liquid ratio is held constant and cooking time is taken as the independent variable, the fuzzy sensory score varies within the range of 55–65 min of cooking time, exhibiting an initial increase followed by a decrease. Conversely, when the cooking time is held constant and the material-to-liquid ratio is taken as the independent variable, the fuzzy sensory score likewise shows an initial increase followed by a decrease as the material-to-liquid ratio varies within the range of 0.64–0.8. Comparing the ranges of variation for material-to-liquid ratio A and cooking time B in the 3D plot, it is evident that the surface contour corresponding to cooking time exhibits a steeper slope than that corresponding to the material-to-liquid ratio; thus, cooking time exerts a more pronounced influence on the fuzzy sensory score.

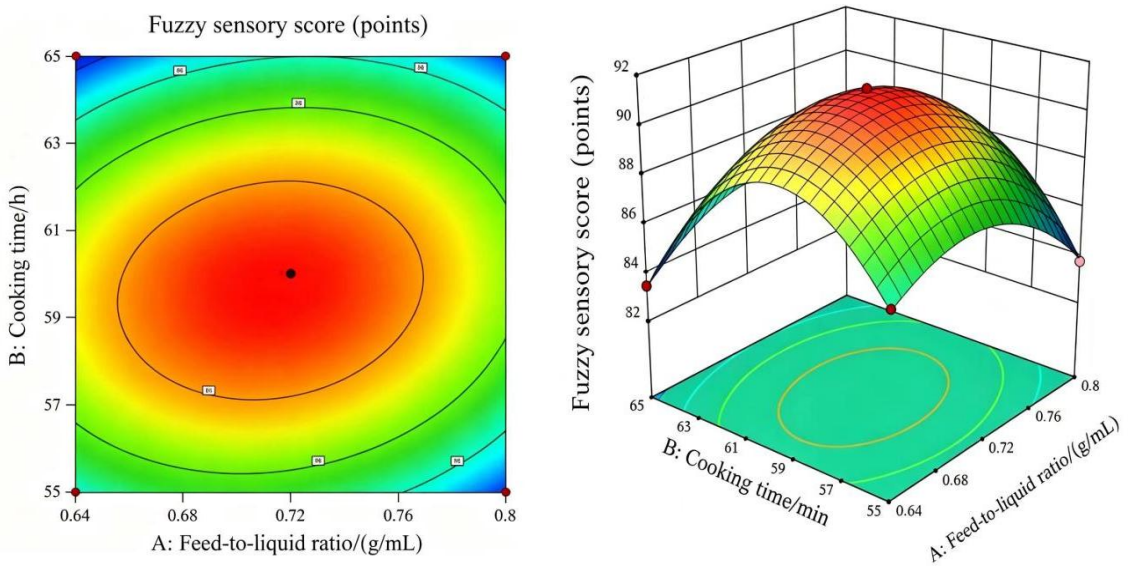


Figure 4. Response surface plot illustrating the effect of the interaction between the material-to-liquid ratio (A) and cooking time (B) on the fuzzy sensory scores.

(2) Comparison of the Significance of the Interaction Between The Material-To-Liquid Ratio And Soaking Time on Fuzzy Sensory Scores

By analyzing the interaction response surface plot of material-to-liquid ratio A and immersion time C in Figure 5, it was observed that when the material-to-liquid ratio was held constant and immersion time was used as the independent variable, the fuzzy sensory score exhibited a subtle initial increase followed by a decrease as immersion time varied within the range of 24–72 h; the 3D plot showed slight fluctuations, while the surface plot displayed relatively gentle variations. This indicates that the influence of immersion time C on the fuzzy sensory score was not particularly significant. Conversely, when the immersion time was held constant and the material-to-liquid ratio was used as the independent variable, the fuzzy sensory score demonstrated a clear initial increase followed by a decrease as the material-to-liquid ratio varied within the range of 0.64–0.8. In this scenario, the 3D plot exhibited substantially greater variation, and the curvature of its surface plot was markedly steeper than that observed in the immersion time scenario. Analysis revealed that, under this two-factor interaction experiment, the effect of the material-to-liquid ratio A on the fuzzy sensory score was greater than that of immersion time C.

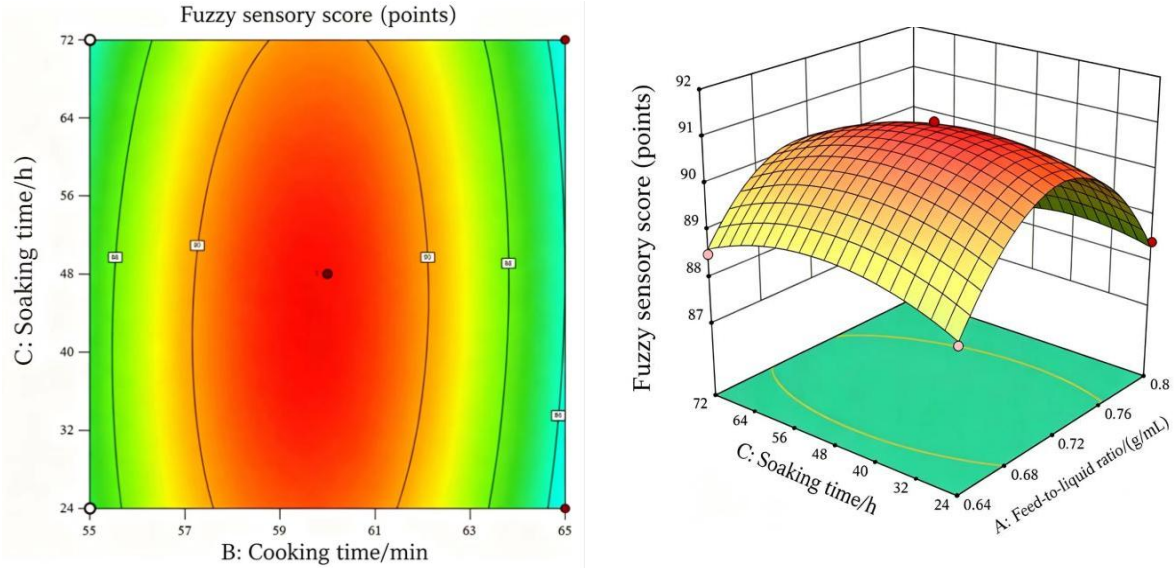


Figure 5. Response surface plot illustrating the effect of the interaction between the material-to-liquid ratio (A) and immersion time (C) on the fuzzy sensory scores.

(3) Comparison of the Significance of the Interaction Between Cooking Time And Soaking Time on Fuzzy Sensory Scores

By analyzing the interaction response surface plot of boiling time (B) and soaking time (C) in Figure 6, the following conclusions were drawn: when the boiling time was held constant and soaking time was used as the independent variable, as soaking time varied within the range of 24–72 h, the fuzzy sensory score exhibited a subtle initial increase followed by a decrease; the 3D plot showed slight fluctuations, while the surface curve displayed relatively steady oscillations. This indicates that the influence of soaking time (C) on the fuzzy sensory score was not particularly significant. Conversely, when the soaking time was held constant and boiling time was used as the independent variable, the fuzzy sensory score demonstrated a clear initial increase followed by a decrease as boiling time varied within the range of 55–65 h. In this scenario, the 3D plot exhibited pronounced variations, and the curvature of the surface curve assumed an "arched" shape, which was significantly steeper than that observed on the soaking time side. Consequently, in this two-factor interaction experiment, the effect of boiling time (A) on the fuzzy sensory score was greater than that of soaking time (C).

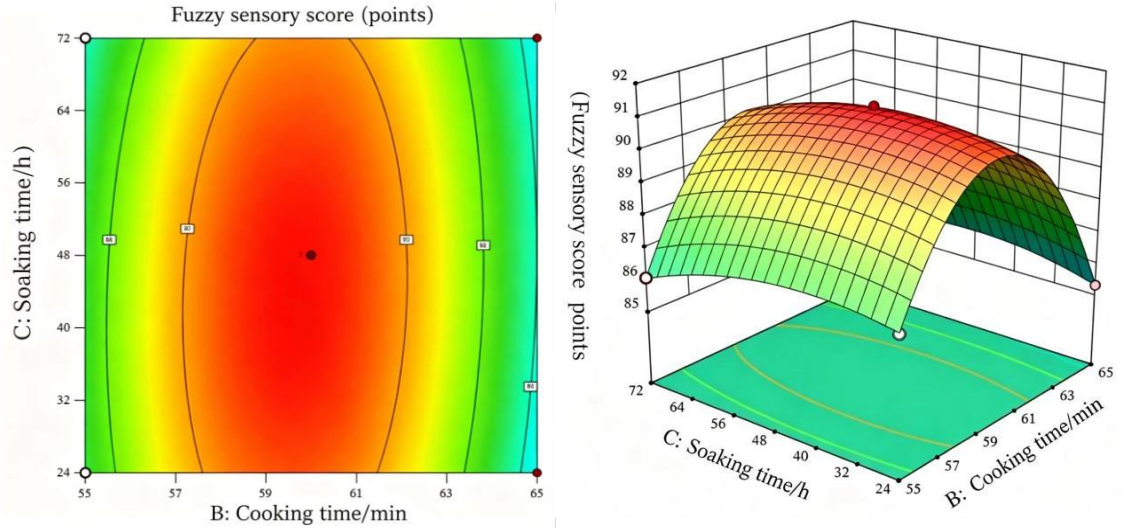


Figure 6. Response surface plot illustrating the effect of the interaction between cooking time (B) and soaking time (C) on the fuzzy sensory scores.

3.3.4. Optimal Process Combination

Using Design-Expert 13.0.5 software, we performed quadratic regression analysis on the response surface to determine the optimal simulated process parameters for a 3-factor, 3-level experimental design: material-to-liquid ratio of 0.713093 g/ml, boiling time of 59.6086 min, and soaking time of 43.9771 h. Through the screening system and experimental design of this model, the final fuzzy sensory score for the flavor of cowhide reached 91.1146 points.

3.3.5. Modelling Verification

To better align with practical production conditions, slight adjustments were made to the optimal process parameters: a liquid-to-solid ratio of 0.70 g/ml, a boiling duration of 60 min, and a soaking duration of 45 h. Subsequently, three parallel experiments were conducted, yielding a fuzzy mathematical score of 90.36 for the flavorized cowhide, which overall matched the predicted value, thereby confirming the rationality of this prediction.

3.4. Microstructural Analysis By Scanning Electron Microscopy

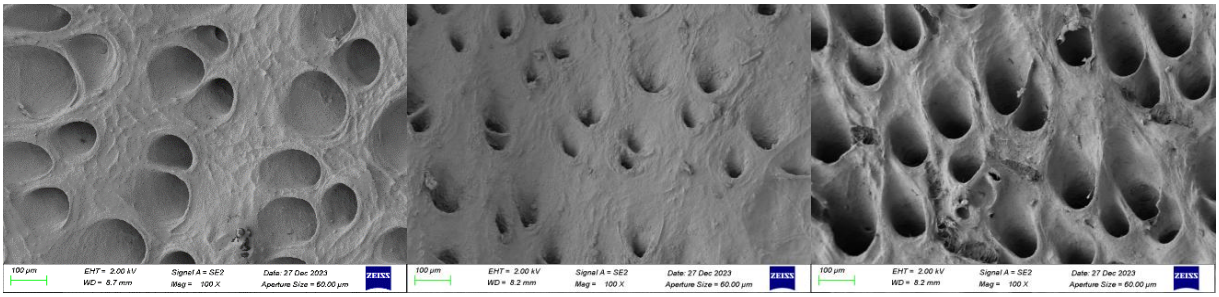


Figure 7. Scanning electron micrographs of raw, cured, and flavored cowhide products (100×)

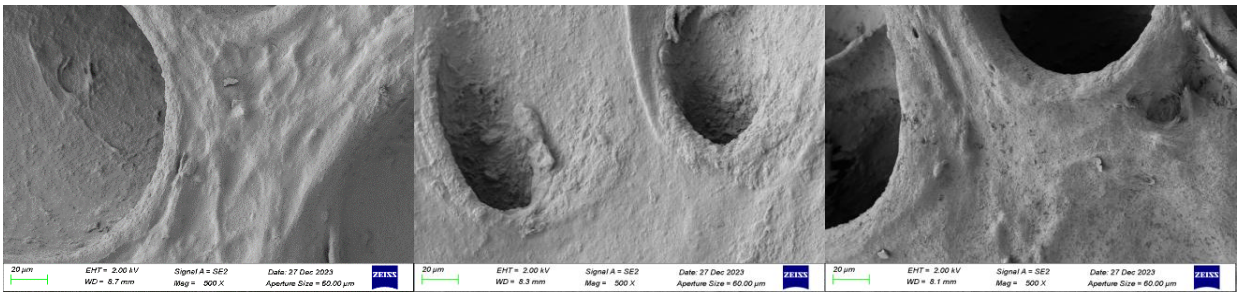


Figure 8. Scanning electron micrographs of raw, cured, and flavored cowhide products (500×)

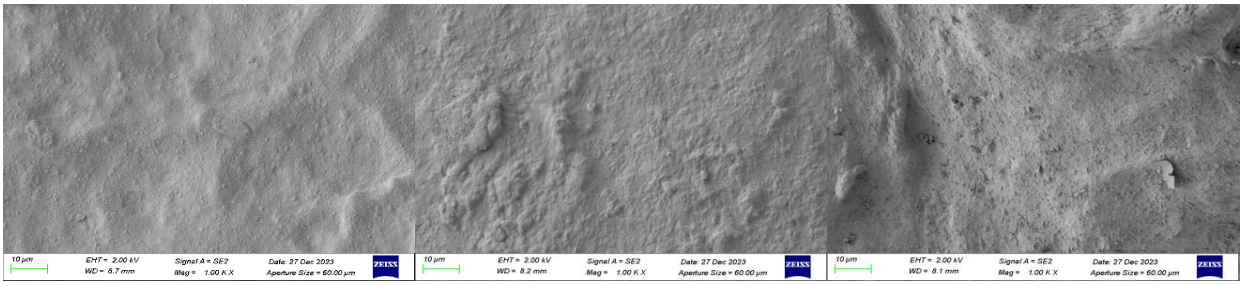


Figure 9. Scanning electron microscopy images of raw, cured, and flavored cowhide products (1,000×)

As shown in Figures 7–9, at a 100× magnification, raw cowhide exhibits a relatively regular and uniformly sized pore structure. After undergoing the boiling treatment, the pore size of cured cowhide decreases, the pore depth becomes shallower, and the inter-pore spacing widens. In contrast, the pore structure of flavored cowhide transforms into an irregular honeycomb-like pattern, with cracks appearing in certain regions. At 500× magnification, the microstructural details of raw cowhide become more pronounced: the pores are larger with relatively well-defined edges, and the surface displays fine fibrous textures that exhibit a certain degree of order, reflecting the structural characteristics of the natural collagen fibers in cowhide. In cured cowhide, the pore edges become blurred, the surface fibrous textures are less distinct, and the overall structure appears denser. The microstructure of flavored cowhide exhibits a rough, uneven surface, with small granular particles observable adhering to the surface. At 1000× magnification, the surface of raw cowhide appears relatively smooth, though subtle undulations and a few minute granular particles remain visible; these particles may represent naturally occurring protein aggregates or impurity components within the cowhide tissue. The surface of cured cowhide displays a complex microstructure characterized by fine grooves, wrinkles, and localized micro-protrusions. Flavored cowhide, meanwhile, exhibits dense protrusions and crack networks, accompanied by a significantly increased surface roughness.

The microstructural characteristics of raw cowhide can be attributed to the maintenance of a triple-helix structure by collagen fibers. This highly ordered triple-helix architecture lays the foundation for the structural and functional stability of collagen [13]. Such a structure enables the preservation of intrinsic mechanical strength and toughness, allowing the hide to effectively resist external physical stresses—such as friction and tensile forces—within the living animal. In contrast, the microstructure of cured cowhide can be analyzed at the molecular level: elevated temperatures induce thermal denaturation of the collagen in the hide. During heating, the hydrogen bonds between the three helical peptide chains of collagen weaken, leading to gradual unwinding of the helices, molecular extension, and increased protein disorder [14]. These structural alterations may modify intermolecular distances and interaction patterns, resulting macroscopically in pore contraction and interstitial widening. Concurrently, the newly formed surface topography following protein rearrangement may contribute to an increase in the surface roughness of cured cowhide. These microstructural changes not only create more favorable conditions for the penetration and adsorption of flavor compounds during subsequent processing stages but also exert a certain influence on the textural properties of cured cowhide. The formation of this complex microstructure in flavored cowhide may, on one hand, be influenced by the addition of specific additives during processing. For instance, the addition of NaCl reduces the gel strength of collagen; this occurs because sodium ions can directly disrupt the hydration bonds and hydrogen bonds within the collagen solution, or alternatively, alter the water microenvironment surrounding the gelation zones, thereby compromising the stability of collagen crosslinking sites and ultimately diminishing the gel strength of the collagen solution [15]. Sucrose, a polyhydroxy compound, enhances the stability of intermolecular hydrogen bonds in collagen molecules; however, its effect on gel strength is far less pronounced than that of NaCl [16]. Consequently, this process imparts a richer and more layered mouthfeel to cowhide. On the other hand, various processing steps during production—such as soaking and vacuum packaging—may exhibit synergistic effects, further accelerating structural evolution. This distinctive irregular honeycomb structure, along with its crack network, significantly increases the specific surface area of flavored cowhide, enabling it to adsorb and retain flavor compounds more effectively. During consumption, the chewing action gradually disrupts this structure, leading to the progressive release of flavor compounds and thereby providing consumers with a unique textural and flavor experience.

3.5. Textural Analysis

Textural analysis was conducted on flavored cowhide using multiple parameters, including hardness, adhesiveness, elasticity, and chewiness; the results are presented in Table 8. Further analysis of these textural data yielded the correlations among the various textural parameters of cowhide, as shown in Table 9. Among the parameters, hardness exhibited the strongest correlation with both adhesiveness and chewiness; adhesiveness showed strong correlations with elasticity and resilience, as well as with cohesion; cohesion demonstrated strong correlations with adhesiveness; elasticity showed strong correlations with chewiness; and adhesiveness also exhibited strong correlations with chewiness.

Table 8. Textural test results

Skin type	hardness	stickiness	Reactive property	cohesion	elasticity	adhesiveness	Chewability
Raw cowhide	41666.935	69359.994	51.307	1	99.949	41666.937	41645.533
Treated cowhide	38671.213	104121.163	22.946	0.823	98.232	32326.615	32025.533
special flavour cowhide	8579.91	-1.07	89.3	0.97	98.6	8316.84	8204.12

Table 9. Texture correlations of flavored beef

	hardness	stickiness	Reactive property	cohesion	elasticity	adhesiveness	Chewability
hardness	1						
stickiness	0.041	1					
Reactive property	0.247	-0.958	1				
cohesion	0.267	-0.952	1.000*	1			
elasticity	0.691	0.751	-0.53	-0.512	1		
adhesiveness	0.982	-0.15	0.427	0.446	0.54	1	
Chewability	1.000*	0.065	0.224	0.244	0.708	0.977	1

The closer the absolute value of the correlation coefficient r is to 1, the stronger the correlation; if r> 0, the two indicators exhibit a positive correlation; if r <0, the two indicators exhibit a negative correlation.

In the study of flavored cowhide, a precise evaluation system was established using fuzzy mathematical sensory evaluation, encompassing five key aspects: color, flavor, firmness, elasticity, and texture. Based on these five criteria, sensory evaluations were conducted to determine the optimal processing parameters, yielding a sensory score of 90.36. Furthermore, texture analysis results demonstrated that each texture indicator was closely correlated with these sensory evaluation metrics.

In sensory evaluation, the hardness of flavored beef skin directly influences the chewing experience. Excessive hardness can make chewing more strenuous, deviating from the ideal state of "compact texture and sufficient chewiness"; this not only reduces the individual "hardness" score but may also adversely affect overall mouthfeel, thereby lowering scores for flavor, springiness, and chewiness, which in turn leads to a decline in the overall sensory score. Conversely, moderate hardness can elevate the "hardness" score, contributing to an increase in the overall score. Adhesiveness exhibits a negative correlation; its low tendency to adhere to teeth provides a refreshing mouthfeel during sensory evaluation, aligns with the requirement for "moderate chewiness," positively impacts the "springiness" score, and enhances the "flavor" score by not interfering with flavor release—collectively driving up the overall sensory score. Good recoverability, cohesion, and elasticity indicate a stable product structure with excellent resilience, enabling the product to rapidly regain its original shape during chewing. This aligns with the criteria of "compact texture and superior chewiness," boosts the "springiness" score, and, due to its dense structure, ensures a solid mouthfeel, which is favorable for the "tissue structure" score. Overall, these characteristics help elevate the comprehensive sensory score. High values for adhesiveness and chewiness, however, imply greater chewing difficulty; for consumers who prefer foods with a chewy texture, this can enhance scores for "hardness" and "springiness," thereby accentuating product distinctiveness and raising the overall sensory score. The interrelationships among these textural indicators and sensory evaluation metrics provide critical insights for understanding the quality characteristics of flavored beef skin, while also offering strategic guidance for process optimization and quality enhancement.

3.6. Fourier Transform Infrared Spectroscopy Analysis

3.6.1. One-dimensional Infrared Spectrum

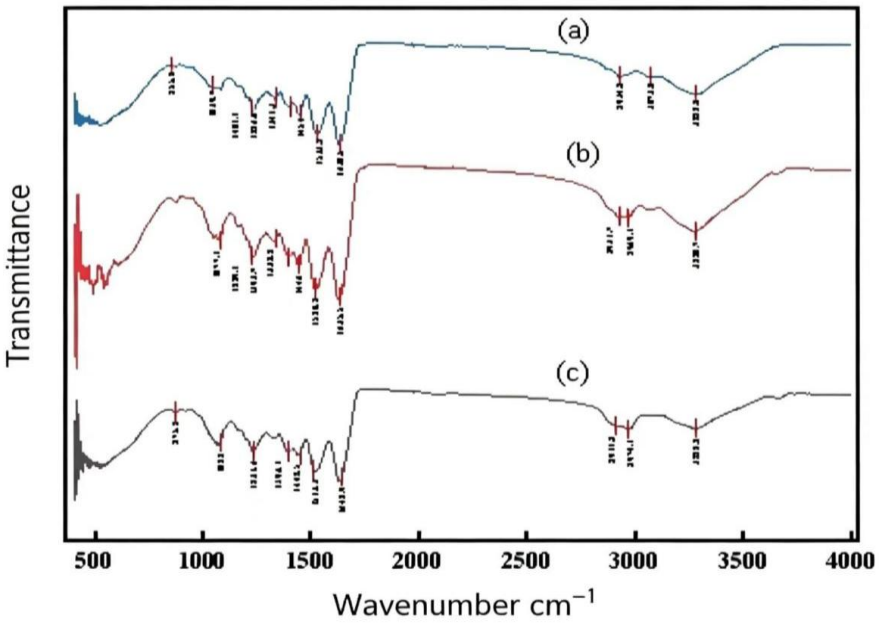


Figure 10. shows the one-dimensional infrared spectra of flavored cowhide, cured cowhide, and raw cowhide, from top to bottom.

According to the analysis of the one-dimensional infrared spectroscopy spectra in Figure 10, the characteristic chemical groups in raw cowhide are as follows: the broad peak near 3300 cm^{-1} corresponds to the N–H stretching vibration of collagen [17, 18] and the O–H stretching vibration of water; the peaks at 1642 cm^{-1}, 1513 cm^{-1}, and 1231 cm^{-1} are respectively assigned to the amide I band (C=O stretching), the amide II band (N–H bending and C–N stretching), and the amide III band (C–N stretching and N–H bending), indicating that raw cowhide primarily consists of the triple-helix structure of collagen—a hallmark feature of collagen. Raw cowhide also exhibits stable intermolecular crosslinks, resulting in a higher degree of collagen structural regularity [18, 19]. The CH_2/CH_3 bending vibration at 1448 cm^{-1} and the C–O stretching peak at 1082 cm^{-1} may be associated with the side chains of collagen. After poach treatment, collagen fibers in cooked cowhide undergo thermal denaturation, leading to weakened hydrogen bonding [14], manifested as a decrease in the intensity of the N–H/O–H peaks (from 3288 to 3280 cm^{-1}), a blue shift of the amide I band to 1635 cm^{-1}, and the emergence of a new C–O peak near 1077 cm^{-1}, which may be related to the short-chain structures of collagen hydrolysis products. After immersion in the seasoning solution (containing salt, sugar, chili oil, etc.), the infrared spectra of flavored cowhide exhibit significant changes: the ortho-substitution peak at 855 cm^{-1} may indicate the adsorption of aromatic compounds from the chili oil; the enhanced CH_2/CH_3 peak at 2934 cm^{-1} may be associated with yeast strains present in the yeast extract [20, 21]; and the intense C-O peak at 1039 cm^{-1} may originate from the introduction of fatty acids from the chili oil [22]. The C–H stretching vibration of aromatic rings at 3073 cm^{-1} serves as a key diagnostic feature of aromatic compounds. Concurrently, the penetration of salt ions may lead to shielding of hydrophilic groups in collagen (resulting in further attenuation of the O–H peak), while the blurring of the amide bands (1540–1640 cm^{-1}) may reflect the interference of small flavor molecules with the vibrations of the collagen backbone. In summary, cooked cowhide primarily undergoes collagen denaturation, whereas flavored cowhide, following a series of processing steps, acquires aromatic compounds, thereby enriching its aromatic profile.

3.6.2. Two-dimensional Synchronous Infrared Spectroscopic Analysis

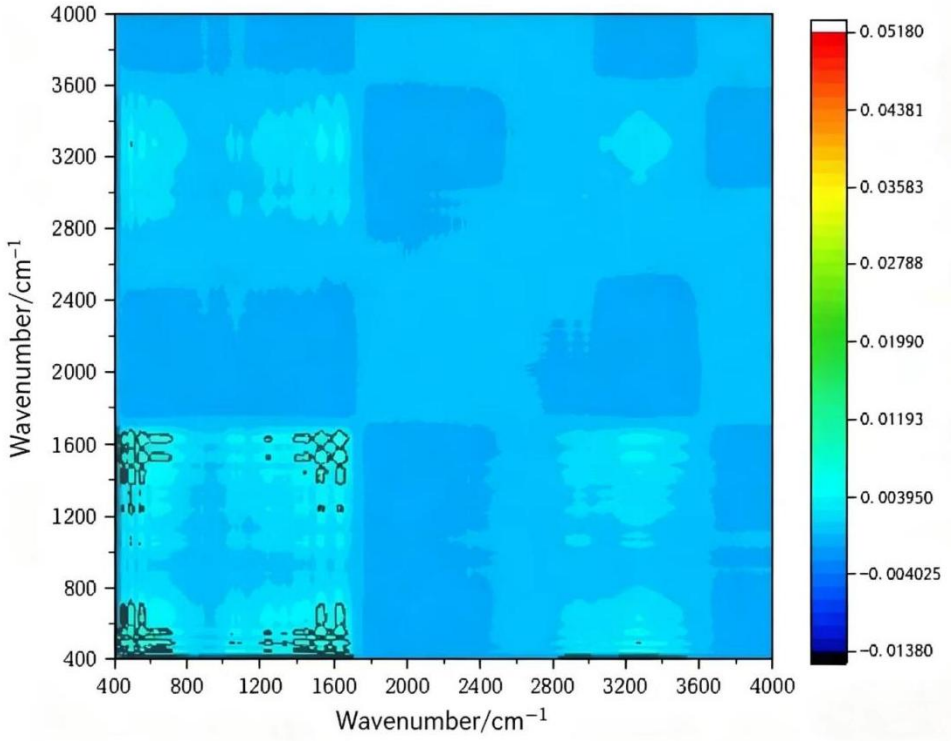


Figure 11. Two-dimensional synchronous infrared spectra of raw, tanned, and flavored cowhide

According to the analysis of the two-dimensional synchronous infrared spectroscopy spectra in Figure 11, the intense absorption bands of raw cowhide in the 1640–1680 cm^{-1} region primarily correspond to the amide I band (C=O stretching vibration) of collagen, while the absorption bands in the 1520–1560 cm^{-1} region (amide II band) corroborate the stability of the hydrogen-bond network within the collagen triple-helix structure [23]. In cooked cowhide, the amide I band absorption bands shift toward lower wavenumbers to the 1625–1635 cm^{-1} range; this redshift indicates that during the boiling process, high temperatures disrupt the hydrogen bonds responsible for stabilizing the collagen triple-helix structure, leading to thermal denaturation of collagen and a reduction in its helical structure [24, 25], thereby altering its infrared absorption characteristics. In flavored cowhide, intense absorption bands appear in the 2800–3000 cm^{-1} region (approximately at 2920 cm^{-1} and 2850 cm^{-1}), which may correspond to the symmetric and antisymmetric C–H stretching vibrations of lipid components derived from chili peppers. In this region, a positive crosspeak is observed with the amide I band, potentially reflecting interpenetration and binding between lipid molecules and denatured collagen; additionally, new crosspeaks observed in the 1000–1200 cm^{-1} region (characteristic vibrations of C–O and polysaccharides) may result from interactions between yeast extract in the soaking solution and collagen structures. Comprehensive analysis demonstrates that the boiling treatment of cooked cowhide disrupts hydrogen bonds, providing a molecular-level explanation for the release of flavor compounds in flavored cowhide.

4. CONCLUSIONS

Building upon a single-factor experiment, this study employed fuzzy sensory scoring combined with response surface methodology to optimize the production parameters for flavored cowhide, yielding the following optimal conditions: material-to-liquid ratio of 0.70 g/ml, boiling duration of 60 min, and soaking duration of 45 h. The sensory score achieved was 90.36 points. Scanning electron microscopy (SEM) images revealed a honeycomb-like microstructure in the cowhide, with increased pore diameter and enhanced surface roughness compared to raw cowhide. Texture analysis demonstrated that the flavored cowhide exhibits high elasticity and low hardness. Fourier transform

infrared spectroscopy analysis indicated that boiling induces thermal denaturation of collagen in the cowhide, providing a foundational basis for its processing. The processed cowhide contains aromatic compounds, imparting a rich aroma. Under this formulation, the cowhide exhibits uniform texture, smoothness, springiness, and superior mouthfeel. These findings contribute to increasing the added value of cowhide and extending its industrial value chain.

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