

Research on the preparation and quality control process of cattle liquid tallow

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ABSTRACT

Liquid butter is a special process with unique flavor, which has strong commercial value in the deep processing of animal fat. In this paper, the preparation of liquid butter was based on centrifugal supernatant as the main raw material, and the process of preparing liquid butter by ultrasonic-assisted transesterification method was used to optimize the preparation of liquid butter by single factor and orthogonal experimental design according to the sensory, freezing point and iodine value of the product. The experimental results showed that the optimal formula of liquid butter was as follows: liquid-to-material ratio of 1:1, ultrasonic temperature of 50°C, and ultrasonic time of 50min. Using this ratio, the sensory score of liquid butter prepared from centrifugal supernatant was 87 points, the freezing point was 22.9°C, and the iodine value was 67.5g/100g. The liquid butter prepared by this process is clear and translucent, with unique flavor, and has certain nutritional and health care effects, which has certain reference value for future application in the field of deep processing of animal fat.

KEYWORDS

liquid butter; transesterification; enzymes; freezing point; iodine number; orthogonal test

1. INTRODUCTION

In the rapid development of today's society, the importance of the deep processing quality of animal fats and fats is becoming increasingly prominent, among which butter has become an indispensable research object in the food industry. Butter refers to the veterinarian examination of healthy cattle after slaughter, take its fresh, clean and intact adipose tissue to refine the oil, the color is light yellow, the taste is delicate, it is a kind of oil with high hardness, rich in fatty acids, triglycerides and fat-soluble vitamins, its nutritional value is high, the fragrance is rich, but also has a wide range of physical and chemical properties and plasticity[1]. Compared with vegetable oil, refined butter has an irreplaceable special flavor and is widely used in the food processing industry[2] due to the high freezing point of butter itself, it quickly forms a solid state at room temperature, and the plasticity of butter is poor, the crispiness is not good, and the saturated fatty acid content of butter is high, which is easy to cause health problems such as hypertension, arteriosclerosis, and coronary heart disease, so it is not suitable as a direct edible oil and cannot solve the market demand[3] therefore, the preparation of liquid butter plays an important role in the processing of animal fats and fats, and liquid butter is modified by some technical methods[1] the main purpose of butter modification[4] is to broaden the application range of animal fat, and its specific modification methods include: transesterification and emulsification methods[5] in the experiment, zhang jinting [6] method added different wetting agent solutions to butter found that the freezing point of the wetting agent solution in liquid butter did not

decrease significantly, and the action capacity was weak, so the experiment was carried out by transesterification method and related experiments according to lipase-assisted treatment and ultrasonic treatment. Lipase is an enzyme that can not only hydrolyze long-chain esters in the aqueous phase, but also catalyze ester synthesis or transesterification in the non-aqueous phase[7] after the preliminary experiment, it was found that one of the two lipases had good stability and had a significant effect during the experiment. During the experiment, ultrasonic treatment attracted much attention for its ease of operation and high product quality[8]in which ultrasonic treatment generated huge mechanical energy and shear forces, resulting in cavitation [[9]surface peeling, and particle fragmentation[10]it increases the solubility of the substrate and enhances the interaction with enzymes to produce a synergistic effect[4]it interacts with the reactive molecules, vigorously shakes and mixes, and higher process efficiency occurs [11]. Transesterification is a directional process catalyzed by lipases with high selectivity, milder reaction conditions, and fewer side reactions[12]which changes the position of fatty acids on triglycerides in response to catalyst enzymes through the rearrangement of fatty acids in the glycerol backbone[13] thus changing the melting and freezing points of the grease. Ultrasonic transesterification is an irreversible chemical change in the composition of fatty acids under the action of a catalyst. After the preparation of butter, the saturation can be reduced, the freezing point can be reduced, the saturated fatty acids will be reduced, and the cholesterol content will be reduced.

In this study, the sensory, iodine value and freezing point were used as evaluation indexes, and the liquid butter was optimized through single factor and orthogonal tests, and the optimal process was obtained through the study and analysis of different factors, and the preparation of liquid butter is of great significance for the future development of animal fat.

2. MATERIALS AND METHODS

2.1. Materials and Instruments

2.1.1 Raw materials

The butter at the refining temperature of 155°C was taken and the supernatant was obtained by centrifugation, and the supernatant was selected as the experimental raw material.

2.1.2 Laboratory apparatus

Table 1-1. Main reagents

Product name	Specification	origin/manufacturer
Lipase	Food grade	chinese mainland
Peanut oil	Food grade	chinese mainland
Cyclohexane	Analyze pure ar	tianjin kemiou chemical reagent co., ltd
Potassium iodate	Analyze pure ar	sinopharm chemical reagent co., ltd
Glacial acetic acid	Analyze pure ar	wuxi jinko chemical co., ltd
Potassium iodide	Analyze pure ar	tianjin damao chemical reagent factory tianjin
Wechsler reagent	Analyze pure ar	kemiou chemical reagent co., ltd
Soluble starch	Analyze pure ar	xilong science co., ltd
Sodium thiosulfate	Analyze pure ar	sinopharm chemical reagent co., ltd

Table 1-2. Instruments and equipment

Serialnumber	Name	Model	Manufacturers
1	Electronic balances	Hc2003	Cixi huaxu weighing instrument co., ltd
2	Electric centrifuge	800	Changzhou surui instrument co., ltd
3	Ultrasonic cleaner	Xo-3200dt	Nanjing xianou instrument manufacturing co., ltd
4	Thermostatic sink	Dc-3010	Changzhou guowang instrument co., ltd
5	Digital thermostatic water bath	Drhh-s6	Shanghai chengjie instrument equipment co., ltd
6	Low-temperature freezer	Bcd-471wdcd	Qingdao haier co., ltd
7	Refrigerator	Bcd-290w	Qingdao haier co., ltd
8	Thermostatically heated magnetic stirrer	Df-101s	Shanghai lichen Bangxi instrument co., ltd

2.2. Preparation Process and Operation Points of Liquid Butter

2.2.1 Process flow of liquid butter

Refining butter -- melting butter -- centrifugation of the supernatant -- mixing of the supernatant with peanut oil --- lipase reaction -- ultrasonic shaking --liquid butter

2.2.2 Key points of operation of liquid butter

Butter refining: butter is refined at a temperature of 155°C, and the flavor is better.

Supernatant: after refining butter, take the supernatant after 5min centrifugation at the same speed.

Preheating: peanut oil[14]is preheated at 37°C for 15 minutes before mixing with the supernatant

Sealing: the mixed solution is placed in front of ultrasound for sealing.

Lipase addition: 1% addition after mixing peanut oil and supernatant[15]

Lipase reaction temperature: since the reaction temperature has a negative effect on enzyme activity at higher temperatures[16]the temperature was chosen at 37°C [17]

Lipase reaction time: 40min

2.2.3 Single factor test for liquid butter process optimization

(1) Liquid-to-material ratio

The mixing ratios of peanut oil[14]and supernatant were controlled as follows: 1:3, 1:2, 1:1, 2:1, 3:1.

(2) Ultrasonic temperature

The ultrasonic temperature is controlled as follows: 50°C, 55°C, 60°C, 65°C, 70°C.

(3)Ultrasonic time

The ultrasound time is controlled separately at: 40min, 50 min, 60 min, 70 min, 80 min.

2.2.4 Orthogonal test of liquid butter process optimization

On the basis of the single factor test, sensory score, freezing point and iodine value were used as evaluation indexes, and the liquid-material ratio (a), ultrasonic temperature (b) and ultrasonic time (c) were selected 3 factors 3 horizontal orthogonal tests, orthogonal tests are shown in table 2-1.

Table 2-1. levels of orthogonal test factors

Level	A: liquid-to-material ratio	B: ultrasonic temperature (°C)	C: ultrasonic time (min)
1	1:2	50	40
2	1:1	55	50
3	2:1	60	60

2.3. Test Methods

2.3.1 Determination of freezing point

Take 15~20g of solid sample in a glass crystallization test tube, and then put it into a water bath to heat it to melt all the reagents, take out the test tube, and put it into a protective tube. Then put in the thermometer and stirrer, start stirring, the reagent gradually cools down, and then the reagent begins to crystallize, the temperature rises rapidly, reaches a certain height and maintains for a certain time, the freezing point temperature at this time is the crystallization point, and the accuracy of the two parallel measurements is $\pm 0.1^{\circ}\text{C}$ [18]

2.3.2 Determination of iodine value:

The iodine value was determined with reference to the "determination of iodine value of animal and vegetable oils and fats" (gb/t 5532-2022): 0.793 g of sirloin oil was accurately weighed and placed in a 250 ml iodine measuring flask, and 20 ml of cyclohexane-glacial acetic acid (1:1, v/v) was added mix the solution, shake well, add 25 ml of wechsler reagent accurately, cover the stopper, shake evenly and place in a dark place for 1 h. After taking out, add 20ml of potassium iodide solution and 100ml of distilled water, shake well, titrate with 0.1 mol/l sodium thiosulfate standard solution, add a few drops of starch solution when titrating until the yellow color of iodine is close to disappearing, shake vigorously while titrating, until the blue color just disappears, that is, the end point of titration, record the volume of consumption standard solution, and do the determination of blank solution at the same time.

The iodine value is calculated by formula:

$$W = \frac{(V_1 - V_2) \times c \times 12.69}{m} \quad (1)$$

During the ceremony:

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

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

V1 – the volume of sodium thiosulfate standard solution consumed by the blank solution in milliliters (ml);

V2 – the volume of sodium thiosulfate standard solution consumed by the sample solution in milliliters (ml);

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

M – the mass of the specimen in grams (g).

2.3.3 Sensory evaluation methods

Referring to the method of feng weiling[19]for sensory evaluation, 10 sensory evaluators were selected from different groups of people to conduct sensory evaluation of liquid butter: color, flavor status, specific evaluation criteria are shown in table 2-2, with a full score of 100 points.

Table 2-2. sensory evaluation standard of liquid butter

Project	Total scores/points	Grading criteria
Color	50	Clear and translucent, with a pale yellow color of 30-50 points; Clear without debris, dark color is 10-30 points; Slight turbidity and loss of light is 0-10 points
Smell	50	No sour smell and undesirable pungent odor, with a unique smell of butter is 30-50 points; There is a slight pungent smell, and the butter flavor is light for 10-30 points; There is a heavier irritating odor, and the unique smell without butter is 0-10 points

2.4. data statistical methods

Correlation analysis and anova were performed using spss statistics 26.0, tabulation using excel 2021 and plotting using origin 2018.

3. RESULTS AND ANALYSIS

3.1 Analysis of Single Factor Results of Process Optimization of Liquid Butter

3.1.1 Determination of liquid-to-material ratio

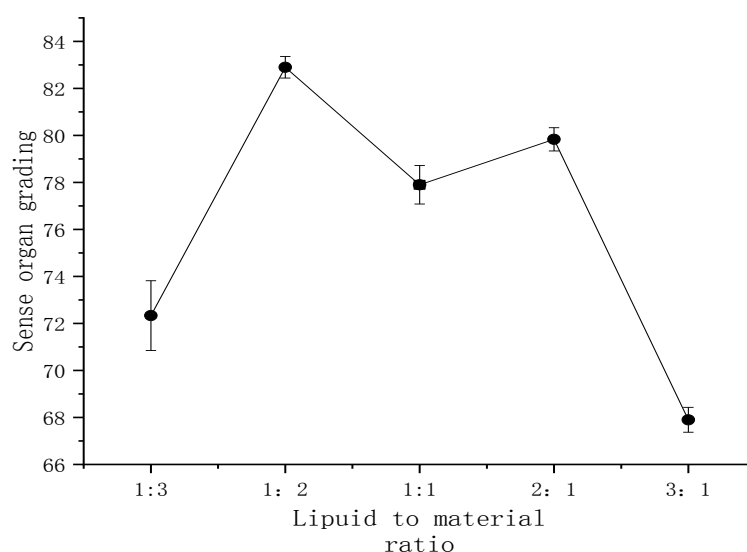


Figure 3-1. effect of liquid-material ratio on sensory scores

As can be seen from figure 3-1, when the liquid-to-material ratio is 1:2, the sensory evaluation is the highest, with a score of 82.9, which is significantly higher than other values. [14]the worse the sensory it is, and the peanut oil [14] with a liquid-to-material ratio of 3:1[14]has a strong taste, which leads to a pungent odor after preparation, and the liquid-to-material ratio of 1:2 is selected for the following test.

3.1.2 determination of ultrasonic temperature

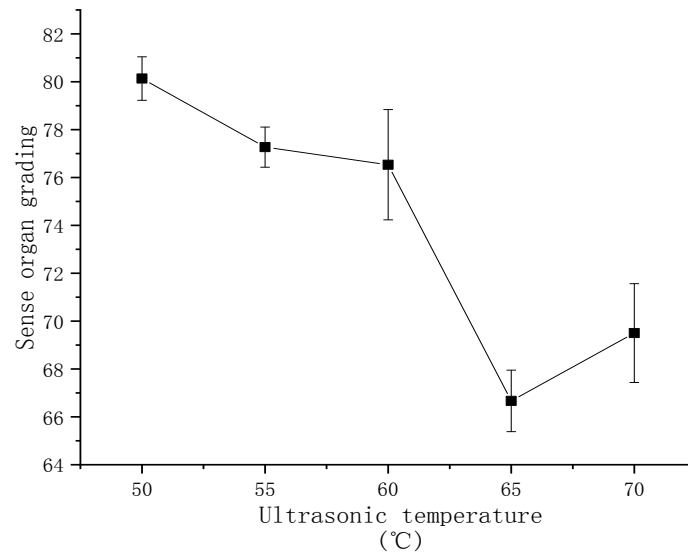


Figure 3-2. effect of ultrasonic temperature on sensory scores

As can be seen from figure 3-2, when other conditions remain constant, the ultrasonic temperature difference is obvious. Through analysis, it was found that the higher the ultrasonic temperature, the more pungent taste appeared, and the sensory evaluation of liquid butter was the highest at the ultrasonic temperature of 50°C. The ultrasonic temperature was selected as 50°C for the following tests.

3.1.3 determination of ultrasound time

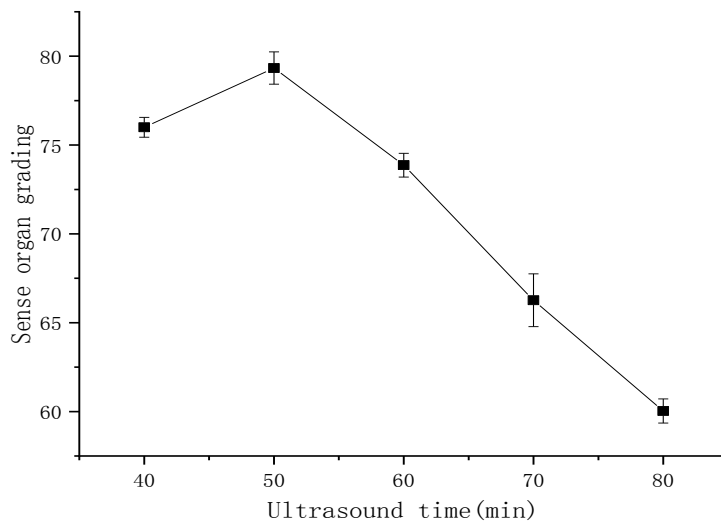


Figure 3-3. effect of ultrasound time on sensory scores

As can be seen from figure 3-3, liquid butter has the highest sensory score when the ultrasonic time is 50 minutes, and it is found that with the longer the ultrasonic time, [20], so that the color and flavor of liquid butter continue to decline, the sensory quality is getting worse and worse, and the flavor of liquid butter has a pungent smell.

3.2 process optimization orthogonal test results of liquid butter

Table 3-4. Orthogonal test table for liquid butte

Experiment number	A	B	C	Empty columns	Sensory score/Divide	Iodine value	Fp(°C)
1	1	1	1	1	74	56.3	25.9
2	1	2	2	2	72.5	59.5	25.2
3	1	3	3	3	68	60.9	24.5
4	2	1	2	3	87	67.4	22.9
5	2	2	3	1	82	67	22.6
6	2	3	1	2	79	68.7	23
7	3	1	3	2	76.3	74.5	24.1
8	3	2	1	3	69.5	77.5	23.8
9	3	3	2	1	72	78.2	20.8
Sensibility Score	k1	71.5	79.1	77.45			
	k2	82.7	74.7	78.54			
	k3	72.6	3	72.22			
	R	11.2	6.1	3			
Iodine Value	k1	58.9	66.1	67.5			
	k2	67.7	68	68.4			
	k3	76.7	69.3	67.5			
	R	17.8	3.2	0.9			
Coagulate Solid Dot	k1	25.2	24.3	43			
	k2	22.8	23.9	45.97			
	k3	22.9	22.8	43.67			
	R	2.4	1.5	2.45			

The sensory results of the orthogonal test in table 3-4 show that the order of action of each factor is a> c> b. The relationship between the level values of each factor is as follows: a2 > a3 > a1, b1 > b3 > b2, c2 > c1 > c3, based on the k-values and the visual comparison, the optimal combination is a2 b1 c2. the order of influence of each factor on liquid butter can be evaluated, among which the proportion of liquid butter has the greatest impact on liquid butter, while the ultrasonic temperature and ultrasonic time order decrease in order. it can be concluded that the optimal preparation process of liquid butter is: the ratio of oil is 1:1, the ultrasonic temperature is 50 °C, and the ultrasonic time is 50min.

Table 3-5. analysis of variance of orthogonal test results with sensory score as evaluation index

Source of variance	Sum of squares of deviations	Degree of freedom	F-number	P-value	Distinctiveness
A	227.242	2	88.230	0.011	*
B	59.642	2	23.157	0.041	*
C	13.609	2	5.284	0.159	

Note: "*" indicates a significant impact on the results (p<0.05). The same applies hereinafter

Table 3-5 shows that the analysis of variance with sensory score as the evaluation index found that a (liquid-to-material ratio) had a significant effect on the sensory aspects of liquid butter (p<0.05), b

(ultrasonic temperature) had a significant effect on the sensory aspects of liquid butter ($p < 0.05$), and the ultrasonic temperature was lower and the liquid butter had better sensory effects.

Table 3-6. analysis of variance of orthogonal test results with iodine value as evaluation index

Source of variance	Sum of squares of deviations	Degree of freedom	F-number	P-value	Distinctiveness
A	477.069	2	145.349	0.007	*
B	15.582	2	4.747	0.174	
C	1.562	2	0.476	0.678	

Table 3-6 shows that the analysis of variance with iodine value as the evaluation index found that a (liquid-to-material ratio) had a very significant effect on the sensory aspect of liquid butter ($p < 0.05$), and the higher the iodine value of liquid butter measured with the higher the amount of peanut oil[14]and the higher the unsaturated fatty acids.

Table 3-7. analysis of variance of orthogonal test results with freezing point as evaluation index

Source of variance	Sum of squares of deviations	Degree of freedom	F-number	P-value	Distinctiveness
A	10.896	2	7.096	0.124	
B	3.749	2	2.441	0.291	
C	2.442	2	1.590	0.386	

Table 3-7 showed that the anova with freezing point as the evaluation index found that the three had no significant effect on the sensory aspects of liquid butter ($p > 0.05$). The reason why the effect of the three effects was not significant in the experimental process was that under the ultrasonic assistance during the transesterification process, a large number of saturated fatty acids in liquid butter were precipitated through the oscillation of ultrasonic, and the stratification phenomenon occurred and white precipitate was precipitated, among which the white precipitate contained various fatty acids, resulting in a significant reduction of the freezing point.

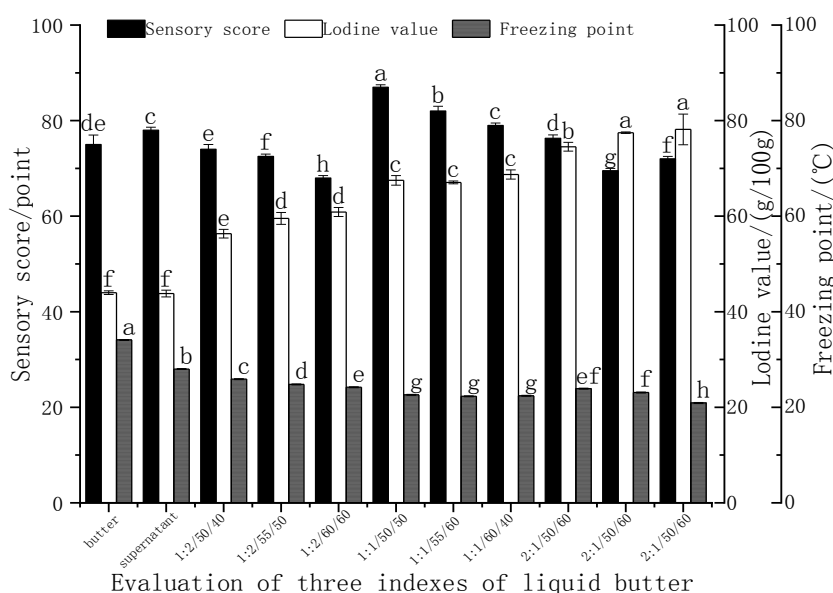


Figure 3-4. evaluation of three indicators of liquid butter

Note: 1:1/50/50 - liquid-material ratio: 1:1/ultrasonic temperature 50°C/ultrasonic time 50min

As shown in figure 3-4, the sensory score, iodine value and freezing point of butter and supernatant and liquid butter were compared with each other. The iodine value of butter was 43.98 g/100 g, while the iodine value of the supernatant was 43.81 g/100 g[21]centrifugation, some of the polyunsaturated

fatty acids are in a liquid state, and the saturated fatty acids accumulate at the bottom as crystal particles, resulting in a decrease in the iodine value of the supernatant. The freezing point of butter was determined at 34.1 °C, while the freezing point of the supernatant after centrifugation was determined at 28 °C, and the freezing point of the supernatant after centrifugation was significantly different, and the freezing point of the supernatant after centrifugation decreased, because butter is a mixture of various fatty acids and contains high saturated fatty acids, which will coagulate at low temperatures[22] during centrifugation, a small amount of saturated fatty acids was precipitated, resulting in a significant decrease in the freezing point of the supernatant compared with butter.

According to the above analysis, it was found that the impurities of the supernatant after centrifugation were reduced, the flavor and color were better, and its freezing point was significantly lower than that of butter, so the supernatant was used as raw material to carry out the test. According to the sensory score of the liquid butter, it was found that the sensory score of 1:1 in the liquid to material ratio in the test was higher than that of other tests and the supernatant, and the ultrasonic temperature in the experimental process was lower than that of other tests, due to the high selectivity and stereo specificity of lipase[23]the experiment, the ultrasonic conditions were mild, so that the aroma composition of the aldehydes in the oil and fat was analyzed, and the refining process had a great influence on the volatile substances in the butter, and there were certain differences in the volatile substances at each stage[24]resulting in a high sensory score of 1:1/50/50 in the test. The 1:2/60/60 period was the worst sensory in the test, during which a pungent flavor was exhibited, in which the ultrasonic temperature and ultrasonic time were high, and the ultrasonic temperature was too high during transesterification and the enzyme was inactivated[25]and the higher the ultrasonic time, the accelerating effect of ultrasound was gradually weakened, resulting in a decrease in lipase activity[26]high temperatures led to a decrease in the selectivity and controllability of autooxidation of liquid butter[27] so the 1:2/60/60 test was less sensory. According to table 4, the effect of liquid-material ratio a on the sensory difference was extremely significant, while the ultrasonic temperature of b had a significant effect ($p < 0.05$). According to the comparison of the iodine value of liquid butter with the iodine value of the supernatant in the experiment, it was found that with the increase of the proportion of peanut oil, the higher the iodine value of liquid butter, the higher the polyunsaturated fatty acids[28]and the hydrolysis of natural substrate oil catalyzed by lipase during transesterification enriches unsaturated fatty acids in liquid butter[29]. Among them, it was found that the higher the ultrasonic temperature and ultrasonic time, the higher the iodine value, the higher the unsaturated fatty acids, and the oxidation rate of the oil was closely related to the unsaturation of the oil, and peanut oil contained more unsaturated fatty acids than butter, and the stability of these unsaturated fatty acids was worse than that of saturated fatty acids at higher temperatures, and they were more likely to be oxidized, resulting in poor sensory deterioration of liquid butter[30] according to table 5, the analysis of variance of iodine value showed that the liquid-to-material ratio of a had a significant effect ($p < 0.05$), which proved that peanut oil was added to the supernatant as vegetable oil and had a strong transesterification effect in liquid butter. According to table 6, the analysis of variance at freezing point showed that the liquid-material ratio, ultrasonic temperature and ultrasonic time had no significant effect on liquid butter ($p > 0.05$).the reason why the effect of the three effects was not significant in the experimental process was that under the ultrasonic assistance during the transesterification process, a large number of saturated fatty acids in liquid butter were precipitated through the oscillation of ultrasonic, and the stratification phenomenon occurred and white precipitate substances, in which the white precipitate substances contained various fatty acids, resulting in a significant reduction of freezing point. Finally, after a multi-faceted comprehensive analysis of single factor and orthogonal tests, the final optimized formula was mainly based on the senses: the liquid-material ratio was 1:1, the ultrasonic temperature was 50 °C, and the ultrasonic time was 50min.

3.3 Validation Experiments

According to the comprehensive evaluation of multiple aspects of single factor and orthogonal test, the comprehensive analysis and selection mainly took the senses as the final optimization formula: liquid-material ratio 1:1, ultrasonic temperature 50°C, ultrasonic time 50 min. Through the verification experiments of the above process, the sensory evaluation score of the optimal process for the determination of liquid butter was 87 points, the iodine value was 67.5g/100g, and the freezing point was 22.9°C.

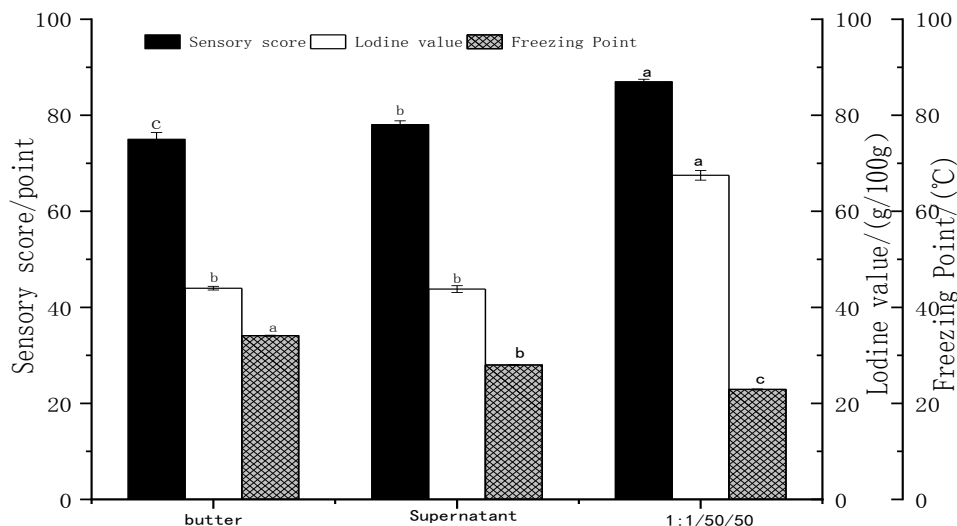


Figure 3-5. evaluation of three indicators of liquid butter

As shown in figure 3-5, the optimal process of butter, centrifugation supernatant and liquid butter was compared with sensory, iodine value and freezing point, and it was found that 1:1/50/50 was the best process for liquid butter preparation, and its liquid-to-material ratio was 1:1ultrasonic temperature 50°C, ultrasonic time 50min compared with butter and centrifugation supernatant after the sensory color and flavor better, sensory up to 87 points, transesterification process butter, centrifugation supernatant and comparison of iodine value, the optimal process liquid butter iodine value than butter and supernatant due to the addition of liquid material ratio a significantly increased, its higher iodine value, indicating that its polyunsaturated fatty acids are high, the comparison shows that the liquid butter process transesterification is significant. According to the comparison of the freezing point, it is found that the freezing point of the optimal process decreases significantly.

4. CONCLUSION

In this experiment, the supernatant after butter centrifugation was used as raw material, and the effects of liquid-to-material ratio, ultrasonic temperature and ultrasonic time on the sensory score of liquid butter were analyzed by single factor experiment, and then the sensory score, iodine value and freezing point of the product were used as indicators to evaluate the orthogonal test, and the experimental results were determined to be the optimal conditions for liquid butter after anova analysis, that is, the liquid-to-material ratio was 1:1 the ultrasonic temperature is 50 °c, and the ultrasonic time is 50min. The sensory quality of liquid butter produced according to this formula process can be highly evaluated, and the liquid butter produced by this process has certain guidance and reference value for the deep processing and development of animal fat in the future.

ACKNOWLEDGMENTS

R & D Plan Zibo City Integration Program (2021SNPT0007), National Modern Agricultural Technology System in China (CARS-37), the Central Guide Local Science and Technology Development Fund Project of Shandong Province (YDZX2022122), the key research and development Program (Rural Revitalization of scientific and technological innovation boost action plan of Shandong Province (2023TZXD046), Key technology research and development for high-quality cattle tallow green deep processing, sgyhx2023-56, 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.

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