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Preparation and Quality Analysis of Pineapple Red Orange Fresh Beer

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Abstract: To optimize the brewing technology of pineapple red orange fresh beer, a single-factor experimental design was used to investigate the effects of fermentation sugar content, fermentation pressure and fermentation temperature. Based on the single-factor test results, the Box-Behnken test design and response surface analysis were used to optimize the process parameters with the sensory evaluation score as the response index. The results showed that the optimal fermentation conditions were fermentation sugar content of 4.00°P, fermentation pressure of 0.13 MPa and fermentation temperature of 18°C. The pineapple red orange fresh beer produced under this technological condition has the characteristics of mellow wheat flavor and antioxidant properties. The research can provide a new direction and application prospect for the development of the pineapple red orange processing industry.

Keywords: Pineapple, Red Orange, Beer, Saccharification and Fermentation, Response Surface

Introduction

In recent years, the output of pineapples has been increasing, but the development of pineapple products is not high currently and the lack of deep processing technology has caused a certain waste of resources. Studies have shown that pineapple peel is not only rich in dietary fiber, yellow pigments, pectins, and polyphenols (Owoeye *et al*., 2022) but also contains mineral elements such as Zn, Fe, Ca, P, K and other minerals that are beneficial to human health (Villacreces *et al*., 2022). The content of Zn, Fe, and Mn in pineapple skin residue is even much higher than that in the pulp. The deep processing of tropical fruits such as pineapple and red-orange can improve their economic value and contribute to the sustainable development of the pineapple and red-orange industries (Li *et al*., 2022).

The demand for low-alcoholic beverages has been rising with the growing interest of health-conscious consumers (del Marmol, 2019; Zhuo *et al*., 2022). China's beer production has been at the forefront of the world, with broad market prospects. People's demand for the health, taste, and nutrition of beer is also increasing (Wang *et al*., 2020; Huang *et al*., 2020). Fruit beer has the uniquely refreshing taste of beer and the sweet taste of fruit, which can meet the different sensory needs and consumption experiences of consumers. Because of the natural bioactive substances contained in raw materials such as fruits and vegetables, it can reduce the oxidation and toxicity of alcohol to the human body and improve the quality index of beer (Penkina *et al*., 2017). In recent years, there have been studies on adding medlar, prickly thorn, and litchi to beer brewing technology, but there is no report on the effect of adding pineapple red-orange on beer quality (Miao *et al*., 2021, Ducruet *et al*., 2017). There are many types of beer produced in China, but the style is relatively single; the taste tends to be homogenized and the fresh craft beer with pineapple juice and red orange juice to add flavor and nutrition has not been reported. The prior art is mostly juice to prepare beer, which is a beverage between fruit drinks and beer. Meanwhile, due to the unfermented juice, the nutrition and health components have not been fully utilized and cannot reflect the unique fruit fermentation flavor.

Given the shortcomings of the current techniques, a pineapple red orange fresh beer and preparation method are provided. In the brewing process, the unique wheat aroma of beer and the rich aroma of fruit are skillfully integrated to effectively retain the plant polyphenols and other components in pineapple and red-orange, to reduce the oxidative and toxic effects of alcohol on the human body and are expected to become a healthy product with development prospects.

Materials and Methods

Experimental Materials

Pineapple: Purchased in Wal Mart; Concentrated red orange juice: Xiamen Ziguo Food Co., Ltd; Pearson malt,

dried wheat, acidified malt: Belgian Castle Malting Company; oats and coriander seed: Yantai Zhifu Xiliang Food Agency; Magnum hops, Moses hops and flax yellow hops: Barthaas (Beijing) Trading Co., Ltd; S-33 yeast, Fermentis, France; other reagents are domestic Analytical Reagents (AR).

Study Design

Process Flow and Key Points of Operation

The operation process is as Fig. 1. The key points for operation are as follows:

(1) 123 kg Pearson malt, 19 kg charred wheat, 9.6 kg acidified malt, and 4.6 kg oatmeal were crushed and mixed with 620 L water at 53°C. Then calcium chloride was added to obtain 120 g of calcium chloride and the pH was adjusted to 6.2
(2) Three-stage saccharification: The mash was saccharified at 50-53°C for 20-30 min, then at 62-65°C for 45-60 min, and finally at 70-72°C for 15-30 min for the iodine test. After the iodine test was qualified, the filtrate and wheat lees were obtained by heating to 76-80°C and filtering. The wort was obtained by combining the filtrate and the water-washing wheat lees
(3) Preparation of pineapple juice: First took 625 kg of fresh and mature pineapples, washed the dirt on the peel, cut the end, removed the peel, and cut it into pieces to put 250 L of water containing 0.33 kg of citric acid and 1.1 kg of salt soaking for 15 min. Then the pineapple pieces were beaten to form pulp juice and the pulp juice was filtered to obtain 248 kg of pineapple juice
(4) Preparation of red orange juice: Took 17 kg of concentrated red orange juice containing 60°BX sugar and added sterile water to dilute to obtain 78.5 kg of red orange juice with a sugar content of 13°BX
(5) Boiled the wort and added once hops during the boiling process, with the additional amount of 0.5 kg/KL wort; At the end of the boiling, added the coriander seeds ground into a powdered form, with the additional amount of 25 g/KL wort; After boiling, the wort was cycloprecipitated and hops were added twice during the cycloprecipitation process, with the additional amount of 0.5 kg/KL wort. The boiling time was 60-65 min
(6) The wort after the cyclotron precipitation was cooled and oxygenated into the fermentation tank and then S-33 yeast was added for the main fermentation at 18-22°C. When the sugar content of the fermentation broth dropped to 3.4-4.6°P, pineapple juice and red orange juice were added for the first time for post-fermentation at 20-22°C
(7) When the pressure in the fermentation tank rose to 0.14-0.16 MPa and 1.5-1.8 times the main fermentation time, the temperature of the fermentation broth was reduced to 7°C. Under the isothermal and isobaric conditions, pineapple juice and red orange juice were added for the second time to maintain for 24 h; Then the temperature of the fermentation broth was reduced to 5°C to maintain for 24 h; finally, the temperature of the fermentation broth was reduced to 0°C and maintained for 10-14 days to obtain the pineapple red orange fresh beer

Single-Factor Experiment

Taking the sensory evaluation score as the index, the single-factor experiment was conducted according to the following steps (Liu *et al*., 2021):

(1) Fermentation sugar content: The fermentation pressure and temperature were fixed as 0.15 MPa and 20°C respectively. The effects of different fermentation sugar values (3.0, 3.5, 4.0, 4.5, and 5°P) on the physicochemical properties and sensory evaluation scores of finished beer were observed
(2) Fermentation pressure value: The fermentation sugar value and temperature were fixed as 4°P and 20°C respectively. The effects of different fermentation pressure values (0.5, 0.10, 0.15, 0.20, and 0.25 MPa) on the physicochemical properties and sensory evaluation scores of finished beer were observed
(3) Fermentation temperature value: The fermentation sugar content and pressure were fixed as 4°P and 0.15 MPa respectively. The influence of fermentation temperature (10, 15, 20, 25, and 30°C) on the physical and chemical properties and sensory evaluation scores of finished beer was observed

Response Surface Test

According to the results of the single-factor experiment, using the Design Expert 8.0.6 software Box Behnken design, taking the fermentation sugar (°P) A, fermentation pressure (MPa) B and fermentation temperature (°C) C as variables and the sensory evaluation scores as response values, a three-factor, and three-level central combination experiment was designed. Each group was repeated three times to take the average value. The factor levels are shown in Table 1.

Quality Analysis of Pineapple Red Orange Fresh Beer

Determination of alcohol content. According to the National Food Safety Standard Determination of Alcohol Concentration in Alcohol (GB 5009.225-2016, China), Carbon dioxide in the sample was removed by shaking and distilling method. In the determination of the sample solution, the bottle stopper with a thermometer was removed. The water was boiled and cooled to 15°C to fill into a constant density bottle, where the stopper

with a thermometer was inserted (there must be no bubbles in the bottle). Then, it was immediately immersed in a constant water bath at 20.0±0.1℃. After the contents reached 20℃ and remained unchanged for 20 min, the liquid overflowing from the side pipe was quickly sucked out by the filter paper, so that the liquid level of the side pipe was flush with the nozzle of the side pipe. After that, the side hole cover was immediately covered to take out the density bottle and the water on the outer wall of the bottle was dried by the filter paper to weigh immediately (m_1). The water in the bottle was poured out and washed with absolute ethanol, then used ether to blow dry (or dried in an oven). Finally, it was washed repeatedly with sample distillate 3-5 times for filling and filling. According to the above method, it was weighed (m_2). The analysis results are obtained by the following formula. The density (ρ_{20}^{20}) of the sample at 20℃ is calculated according to Eq. (1) and the air floatation correction value (A) is calculated according to Eq. (2):

$$\rho_{20}^{20} = \rho_0 \times \frac{m_2 - m + A}{m_1 - m + A} \quad (1)$$

$$A = \rho_u \times \frac{m_1 - m}{997.0} \quad (2)$$

ρ_{20}^{20} = Density of the sample at 20℃, unit: g/L
ρ_0 = Density of distilled water at 20℃ (998.20 g/L)
m_2 = Mass of density bottle and sample at 20℃, unit: g
m = Mass of a density bottle, unit: g)
A = Correction value of air buoyancy
m_1 = Mass of density bottle and water at 20 ℃, unit: g
ρ_u = Density of dry air at 20℃, 1013.25 hPa (≈ 1.2 g/L)
997.0 = The difference in density between distilled water and dry air at 20℃, unit: g/L

According to the density ρ_{20}^{20} of the sample, refer to Appendix A to obtain the alcohol content, which is expressed as the volume fraction "% vol".

Determination of the original wort color. The degassed specimen was injected into a 25 mm cuvette and then placed in a comparator block to compare with the standard color disc and read directly when the two tones were consistent.

Determination of antioxidant activity (Miao *et al*., 2021; Kawa-Rygielska *et al*., 2019; Minami *et al*., 2021). The DPPH radical scavenging capacity and ABTS cationic radical scavenging capacity are based on Trolox as the standard. The DPPH radical scavenging capacity and ABTS cationic radical scavenging capacity of the sample are expressed by the amount of water-soluble vitamin E contained in each liter of beer (mmol/L).

Sensory Evaluation

The sensory evaluation team composed of 15 professionals, scored the pineapple red orange fresh beer from five aspects, including overall impression, aroma, appearance, flavor, and taste. The scoring criteria are shown in Table 2.

Table 1: Variables and levels used in the box-behnken design

	Single-factor		
Level	A Fermentation sugar content (°P)	B Fermentation pressure (MPa)	C Fermentation temperature (℃)
-1	3.5	0.10	15
0	4.0	0.15	20
1	4.5	0.20	25

Table 2: Sensory evaluation method

Items	Scoring criteria	Scores
Overall impression (30 scores)	Harmonious fruit and wine fragrance and good and natural taste	25-30
	A more harmonious fruit and wine aroma and pure flavor with slight flaws	20-24
	Unharmonious fruit aroma and wine aroma, slightly poor fresh feeling of fruit and dried taste	<20
Aroma (15 scores)	Fruity and mellow wheat fragrance	10-15
	Light fruity and mild wheat fragrance	5-9
	No special fruit fragrance, with a prominent alcohol smell	<5
Appearance (15 scores)	Orange-yellow color, foggy turbidity, white, delicate and lasting foam	10-15
	Uniform color, little suspended impurities, and fine and durable foam without white	5-9
	Uneven color, obvious impurities, and the foam was not white, delicate, or durable	<5
Flavor (25 scores)	Obvious pineapple flavor and orange flavor and unharmonious with the malt mellow flavor, without peculiar smell	10-15
	Light pineapple flavor and orange flavor and harmonious with malt mellow flavor, without peculiar smell	5-9
	Lacking pineapple flavor and orange flavor, unharmonious with malt mellow flavor and with peculiar smell	<5
Taste (15 scores)	Superior wine, pure flavor, clean aftertaste, and strong killing taste	10-15
	Medium wine, pure flavor, clean aftertaste, and strong killing taste	5-9
	Inferior wine, general flavor, clean aftertaste, and weak killing taste	<5

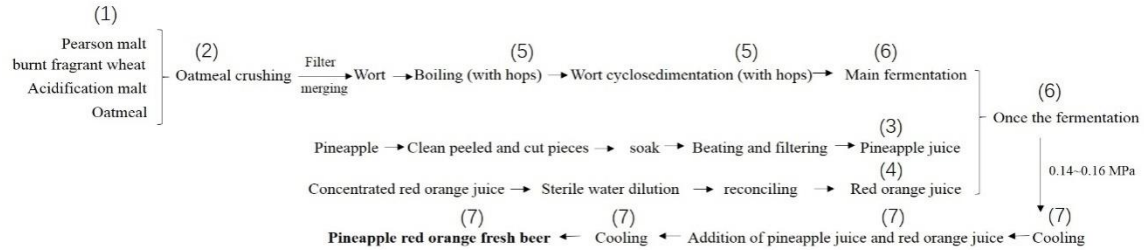

Fig. 1: The operation process

Data Processing

Design Expert 8.0.6 was used for data processing and response surface test design and analysis. All experiments were repeated three times.

Results and Discussion

Single-Factor Experiment Results

Effect of Fermented Sugar Content on Pineapple Red Orange Fresh Beer

The fermentation was carried out under the fermentation temperature of 20℃ and the fermentation pressure of 0.15 MPa and the sugar content of the fermentation broth was detected every 8 h. When the sugar content of fermentation broth dropped to 3.0, 3.5, 4.0, 4.5, and 5.0°P, pineapple juice and red orange juice were added respectively for post-fermentation to explore the effect of different sugar content on pineapple red orange fresh beer. The results of the determination of physical and chemical indicators are shown in Table 3.

Effect of Fermentation Tank Pressure on Pineapple Red Orange Fresh Beer

The fermentation sugar content and fermentation temperature were fixed as 4ºP and 20℃ respectively. The pineapple juice and red orange juice were added into the fermentation tank at the same temperature and pressure for fermentation. After the pressure in the fermentation tank rose to 0.10, 0.15, and 0.20 MPa, as well as 1.6 times the main fermentation time, the influence of the pressure in the fermentation tank on the pineapple red orange fresh beer was explored. The measurement results of physical and chemical indexes are shown in Table 4.

It can be seen from Table 4 that as the fermentation pressure increases, the sensory evaluation result of pineapple red orange fresh beer was the best when the pressure value was 0.15 MPa. The fresh beer prepared has a coordinated fruit flavor and mellow wheat flavor, pure taste, and strong killing taste when the fermentation tank pressure was 0.15 MPa. When the pressure of the fermentation tank was less than 0.10 MPa and more than 0.20 MPa, inappropriate control of the pressure of the fermentation tank will lead to the problem of low killing taste and reduced alcohol thickness. Comprehensively, the fermentation sugar of 0.10 MPa, 0.15 MPa, and 0.20 MPa was selected for the response surface test.

It can be seen from Table 4 that as the sugar increases, the sensory evaluation result was the best when the sugar value was 4.0°P, which had harmonious fruit flavor and mellow wheat flavor, with pure taste and without peculiar smell. When the sugar content of the fermentation broth was greater than or less than 4.0, the taste was weak and the flavor purity was in question due to the inappropriate control of fermentation sugar content and the timing of tank sealing. Comprehensively, the fermentation sugar content of 3.5°P, 4.0°P, and 4.5°P was selected for response surface experiments.

Effect of Fermentation Temperature on Pineapple Red Orange Fresh Beer

The fermentation sugar content and fermentation pressure were fixed at 4°P and 0.15 MPa respectively. The temperature of the main fermentation was controlled from 15℃ to 35℃ to explore the effect of main fermentation temperature on pineapple red orange fresh beer. Table 5 shows that as the fermentation temperature increases, the sensory evaluation of pineapple red orange fresh beer was the best at 20℃. The fresh beer prepared at the main fermentation temperature of 20℃ has a harmonious fruit flavor and mellow wheat flavor, with pure taste, strong killing taste, no fragrance, and a peculiar smell. When the main fermentation temperature was 25°C, the inappropriate temperature of the main fermentation leads to higher alcohols on the high side, rough fat aroma, and heavy yeast taste. Comprehensively, the fermentation sugar of 15℃, 20℃, and 25℃ was selected for the response surface test.

Response Surface Methodology Optimization of Pineapple red Orange Fresh Beer

Optimization of Fermentation Conditions for Pineapple Red Orange Fresh Beer by Response Surface Methodology

Based on the Box-Behnken experimental design, based on the single factor experiment, the processing

technology of pineapple red orange fresh beer was optimized by using the Design Expert 8.0.6 software (Li *et al*., 2021). The three factors, fermentation sugar content (X_1), fermentation pressure (X_2), and fermentation temperature (X_3), were selected to optimize the processing technology of pineapple red orange fresh beer with sensory score (Y) as the response value. The test design and results are shown in Table 6.

Model Establishment and Significance Test

The obtained experimental data were fitted with multiple-regression using Design Expert 8.0.6 software to obtain quadratic multiple-regression equation: $Y = 78.16-1.52A-3.09B+1.56C-3.73AB-4.78AC-2.55BC-4.61A^2-3.78B^2-9.78C^2$.

The analysis of the variance of Table 6 data was performed using Design Expert 8.0.6 (DE 8.0.6) software and the results are shown in Table 7.

Table 3: Physical and chemical index determination result

Fermentation sugar Content (°P)	Original wort concentration (°P)	Alcohol content (%vol)	Chroma (EBC)	Bitterness (IBU)	Sensory score
3.0	12.9	4.8	8.3	11.0	67.10
3.5	13.0	4.9	8.3	10.8	70.90
4.0	13.0	4.9	8.2	10.5	76.40
4.5	13.1	5.0	8.8	9.8	75.00
5.0	13.1	5.0	9.1	9.5	73.30

Table 4: Physical and chemical index determination result

Fermentation pressure (MPa)	Original wort concentration (oP)	Alcohol content (%vol)	Chroma (EBC)	Bitterness (IBU)	Sensory score
0.05	12.5	4.8	7.8	12.1	53.20
0.10	12.8	4.8	7.9	12.0	61.00
0.15	13.0	4.9	8.2	10.5	74.20
0.20	13.1	4.9	8.2	11.4	62.30
0.25	13.2	5.0	8.2	11.5	62.00

Table 5: Physical and chemical index determination result

Fermentation temperature (°C)	Original wort concentration (oP)	Alcohol content (%vol)	Chroma (EBC)	Bitterness (IBU)	Sensory score
15	12.8	4.8	8.2	10.6	72.00
20	13.0	4.9	8.2	10.5	75.40
25	13.1	5.0	9.3	9.8	62.00
30	13.2	5.0	9.3	9.7	60.10
35	13.2	5.0	9.5	9.7	59.80

Table 6: The design and result of the response surface experiment

No.	Fermentation sugar content (X_1)	Fermentation pressure a(X_2)	Fermentation temperature (X_3)	Sensory evaluation score y
1	0	0	0	78.40
2	-1	0	1	70.10
3	0	0	0	78.50
4	0	1	1	62.00
5	1	0	-1	67.00
6	1	-1	0	75.00
7	0	0	0	79.00
8	-1	1	0	72.00
9	0	0	0	76.40
10	0	-1	1	72.00
11	0	1	-1	62.30
12	-1	0	-1	59.10
13	1	1	0	60.10
14	0	0	0	78.50
15	1	0	1	58.90
16	0	-1	-1	62.10
17	-1	-1	0	72.00

Table 7: Variance analysis of response surface method

Source of variance	Sum of squares	freedom	Mean square	F value	P value	Significance
Models	889.32	9	98.81	40.99	0.0001	**
An	18.60	1	18.60	7.72	0.0274	*
B	76.26	1	76.26	31.64	0.0008	**
C	19.53	1	19.53	8.10	0.0248	*
AB	55.50	1	55.50	23.02	0.0020	**
AC	91.20	1	91.20	37.83	0.0005	**
BC	26.01	1	26.01	10.79	0.0134	*
A2	89.29	1	89.29	37.04	0.0005	**
B2	60.16	1	60.16	24.96	0.0016	**
C2	402.73	1	402.73	167.06	<0.0001	**
Residual	16.87	7	2.41			
Misfit term	12.78	3	4.26	4.17	0.1009	
Pure error	4.09	4	1.02			
Total deviation	906.20	16				

Note: $P<0.05$, indicating that the difference is significant, expressed by *; $P<0.01$, indicating that the difference is very significant, expressed by **. A is fermentation sugar content (°P); B is fermentation pressure (MPa); C is fermentation temperature (°C; AB is the interaction between fermentation sugar content and fermentation pressure; AC is the interaction between fermentation sugar content and fermentation temperature; BC is the interaction between fermentation pressure and fermentation temperature

Interaction Analysis

The interaction analysis is shown in Fig. 2 and 3.

From Fig. 2 and 3, it can be seen that the fermentation sugar content has a greater impact on the finished product of pineapple red orange fresh beer, but the interaction is more obvious. The pineapple red orange fresh beer can reach the maximum value near the fermentation sugar of 4.0°P and the fermentation pressure of 0.13 MPa.

It can be seen from Fig. 4 and 5 that the interaction between fermentation sugar content and fermentation temperature is very significant. With the increase of the value, the fermentation sugar content reached the highest value at 4.0°P and the fermentation temperature reached the highest value at 18°C.

It can be seen from Fig. 6 and 7 that the interaction between fermentation sugar content and fermentation temperature is very significant. With the increase in the value, the fermentation pressure reached the highest value at 4.0 MPa and the fermentation temperature reached the highest value at 18°C.

According to the analysis of linear regression results, the best technology and sensory score of pineapple red orange fresh beer were the fermentation sugar value of 3.96°P, the fermentation pressure value of 0.13MPa and the fermentation temperature of 17.88°C and the sensory score was 78.98.

Optimal Process Validation Experiment

To verify the reliability of the optimal process and adapt to the actual operation, the experimental data predicted by the response surface were optimized and modified to determine that the fermentation sugar value was 4.00°P; the fermentation pressure value was 0.13 MPa and the fermentation temperature was 18°C. We conducted three parallel experiments on this formula and conducted a sensory evaluation of the product (Anderson *et al*., 2019; Nardini and Garaguso, 2020). The average value of the three sensory evaluations was 78.98+0.25, close to the predicted value of 78.98, which proved that the model was instructive.

Determination of Antioxidant Activity

There are many antioxidants in beer, such as polyphenols or flavonoids extracted from malt and hops, reductones and melanoids formed during brewing, and glutathione secreted by yeast. Polyphenols in beer mainly come from malt and hops, which are important indexes affecting beer chroma, flavor, taste, and colloidal stability. Red orange pulp is rich in vitamin C, glutathione, total phenols, and flavonoids and pineapple pulp also contains vitamin C and other components. Adding pineapple and red-orange into beer, can enrich the active ingredients in the finished beer and improve the quality and functional activity of beer. The results showed that the DPPH radical scavenging rate and ABTS cation radical scavenging rate in pineapple red orange beer obtained by the optimum technology reached 2.21±0.01 mmol L^{-1} and 4.38±0.02 mmol L^{-1}, respectively.

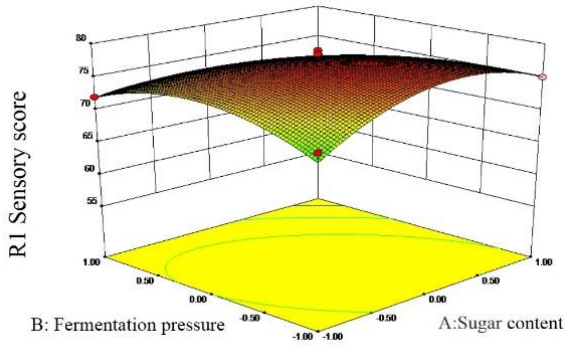

Fig. 2: Response surface diagram of the effects of fermentation sugar content and fermentation pressure on pineapple red orange fresh beer

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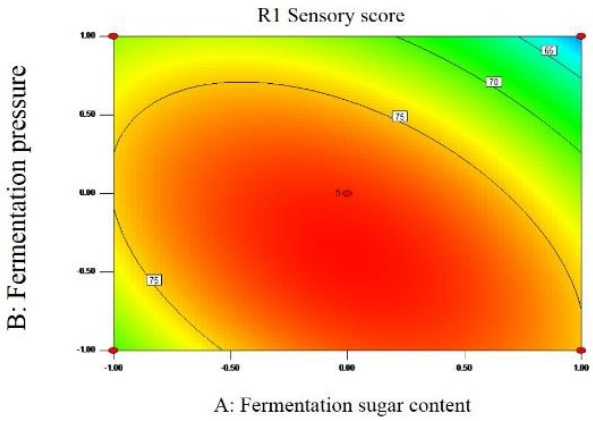

Fig. 3: Contour diagram of the effect of fermentation sugar content and fermentation pressure on the completed product of pineapple red orange fresh beer

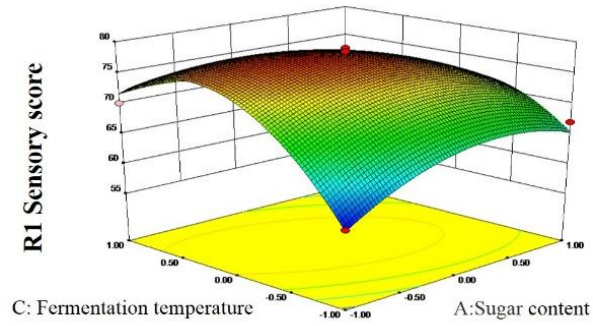

Fig. 4: Response surface diagram of the effects of fermentation sugar degree and fermentation temperature on the finished product of pineapple red orange fresh beer

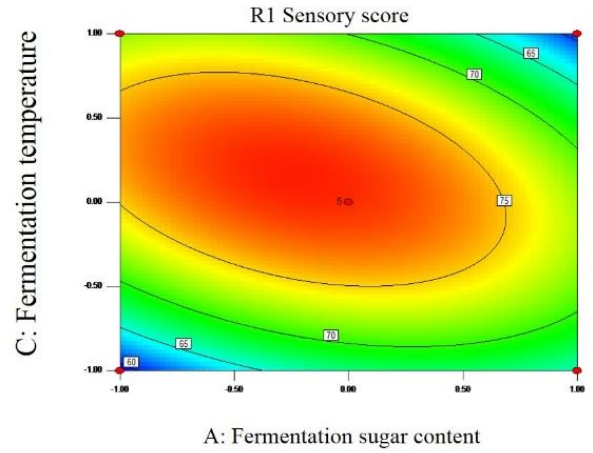

Fig. 5: Response surface diagram of the effects of fermentation sugar degree and fermentation temperature on the finished product of pineapple red orange fresh beer

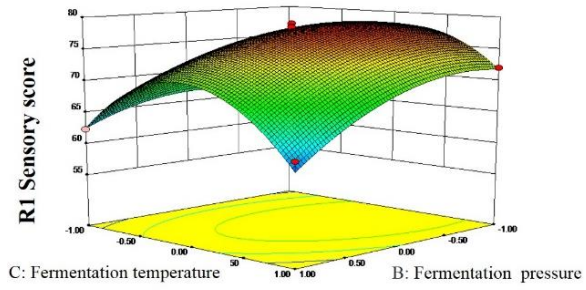

Fig. 6: Response surface diagram of the effects of fermentation pressure and temperature on the finished product of pineapple red orange fresh beer

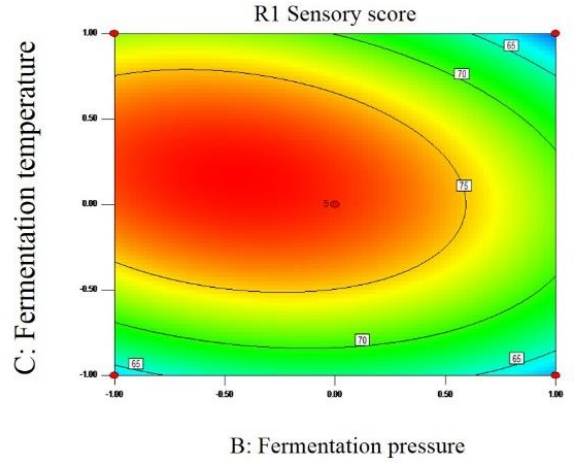

Fig. 7: Response surface diagram of the effects of fermentation pressure and temperature on the finished product of pineapple red orange fresh beer

Conclusion

The study used response surface to optimize the production process of pineapple red orange fresh beer. The best process was that the fermentation sugar value was 4.00°P; the fermentation pressure value was 0.13 MPa; the fermentation temperature was 18°C; the predicted value of pineapple red orange fresh beer was 78.98 and the validation experiment of pineapple red orange fresh beer was 78.98+0.25. DE 8.0.6 software was used to carry out quadratic multiple regression fitting and the model was proved to be valid by significant and insignificant terms of the model. The best process verification experiment showed that the experiment had guiding significance. A better fermentation process to create pineapple red orange beer is presented here and the beer has better sensory values. The fermentation properties, physicochemical properties, antioxidant properties, and sensory properties of pineapple red orange fresh beer were studied. The results showed that the finished beer was pure and harmonious, with a pleasant taste, delicate foam, and characteristic pineapple red-

orange flavor. The research results aim to provide a new direction and application prospect for the development of the pineapple red orange processing industry, as well as a theoretical and practical basis for the follow-up industrial production and the next research. Whether the resulting beer has health benefits requires further studies.

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Author's Contributions

Juan Yang and Zihao He: Designed and performed the experiments, and work.

Daxiang Li and Tao Cheng: Participated to collect the materials related to the experiment.

Juan Yang, Biaoshi Wang and Xiaojun Hu: Designed the experiments and revised the manuscript.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

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