Optimization of fermentation process of papaya sauerkraut using response surface method

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Abstract: Papaya, a tropical fruit was used as the raw material to produce sauerkraut in the study. Three lactic acid bacteria strains isolated from papaya were added to the sauerkraut to facilitate the fermentation of papaya sauerkraut. In the fermentation process, the dynamic changes of total acid in sauerkrauts at different levels of sugar concentration, salt concentration, inoculation and temperature were studied. The response surface method was used to study the effects of changes in multiple factors at the same time. On the basis of "one-variable-at-a-time" approach, the response surface method optimized papaya sauerkraut fermentation process. According to the change of total acid in single factor, 29 experiments were designed by 4×3 factorial central composite design. The optimum fermentation conditions were obtained as follows: sugar at 3.8%, salt at 2.8%, inoculation at 5%, and temperature at 31℃.

Keywords: papaya, sauerkraut, fermentation process, response surface method, lactic acid bacteria, central composite design
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1 Introduction

Sauerkraut, a traditional fermented food in China, is made through fermentation of vegetables such as cabbage and radish seasoned with various spices including red pepper powder, garlic, ginger, and salt. In addition to the original nutrients of vegetables, lactic acid bacteria (LAB) and its metabolites ingested can play an important role in intestinal function, such as modulating immunity, lowering cholesterol and improving lactose intolerance[1,2]. There are many fermentative microorganisms in sauerkraut, especially LAB. The fermenting microflora is dominated by *Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus sake*, and *Lactobacillus brevis*[3].

Eun Kyoung *et al*[4] reported that the consumption of fermented sauerkraut has more beneficial effects than fresh vegetables on metabolic parameters that are related to cardiovascular disease and metabolic syndrome risks in overweight and obese subjects. Cheigh[5] believed that sauerkraut, vegetables fermented with probiotic LAB is a nutritional food that contains high levels of vitamins (ascorbic acid, carotene, B-complex), minerals (calcium, iron, potassium), dietary fiber, and several biologically active components including carotene, capsaicin, chlorophylls, phenolic compounds, ascorbic acid, and lactic acid. These nutrients are considered to be the active agents of sauerkraut's health benefits.

In Hainan Province, a large number of papaya crops could be damaged during the annual typhoon season. Ripe papaya can be eaten directly, while immature papaya tastes bad and therefore has a lower commercial value. Therefore, how to improve the utilization of immature papaya and reduce economic losses is of great significance. In the present study, we used immature papaya as the main material to produce sauerkraut. To the authors' knowledge, there has been no previous research

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on this practice. The study used the response surface methodology to optimize the fermentation process of papaya sauerkraut, which not only laid a theoretical foundation for the industrial production of papaya sauerkraut, but also can improve the utilization rate of quality-inferior papaya.

2 Materials and methods

2.1 Experimental design method

Based on the result of 'one-variable-at-a-time' approach [6], the response surface method (RSM) which takes the total acid as the response was applied to the optimization of the fermentation process, using 4×3 factorial central composite design, described in the central composite design (CCD) of Box and Behnken[7]. The final validation was carried out using the model.

2.2 Starter culture preparation

Lactococcus lactis subsp. *lactis*, *Lactobacillus pentosus* and *Leuconostoc pseudomesenteroides* used for starter culture were isolated from papaya. All strains were cultured in De Man, Rogosa and Sharpe (MRS) broth at 30℃ for 18 hrs and were harvested by centrifugation (Centrifuge, Hettich*UNIVERSAL 32R; Northern fly-domain technology development Co. Ltd beijing; Beijing, China). The harvested cells were washed twice with saline and resuspended in saline for starter inoculation in sauerkraut[8].

2.3 Sauerkraut preparation and sampling

According to the results of the single factor experiment, we determined the fermentation time at three days.

Immature papaya was cut into 10 mm × 10 mm × 10 mm shreds. The papaya shreds were soaked in 0.8% $CaCl_2$ aqueous solution for 1 h, then were removed from the solution and drained, mixed with spices such as pepper, ginger and garlic. The shredded papaya and spices were placed and compacted in jars, according to scheme showed as Table 1. The brine containing different concentrations of salt (2.0%, 4.0% and 6.0%) and sugar (2.0%, 4.0% and 6.0%) was sterilized (Automatic Pressure Steam Sterilizer; GI54DW; Induced micro Instrument Co., Ltd; Xiamen, China) and cooled to room temperature before being transferred into the jars to ensure an anaerobic environment for sauerkraut fermentation. Different inoculation amounts (1.0, 3.0 and 5.0 mL/100 g papaya) of the starter culture were inoculated into different jars and then the jars were incubated at different temperature (20℃, 30℃, 40℃) for three days for the fermentation of the sauerkraut[3]. Three repetitions were collected from each treatment. The pH value of the brine from sauerkraut with different treatments was measured by using pH meter (PHS-3C; Shanghai Precision Scientific Instrument Co. Ltd, Shanghai, China).

2.4 Chemical analysis

Acidity is an important flavor component of sauerkraut and is highly variable since acids are major end-products from the sauerkraut fermentation. Titratable acidity expressed as percent (%) lactic acid, was determined by titration with 0.05 mol/L NaOH with phenolphthalein (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) as an indicator[9]. Total sugar contains reducing sugar and non-reducing sugar. Total sugar measured in this study was mainly the total reducing sugar that came from the hydrolysis of starch polysaccharides and oligosaccharides[9]. Total sugar was determined by the method of Dinitro Salicylic Acid Reagent (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Nitrite levels of sauerkrauts were determined by the method of using sulfanilamide and N-(1-naphthyl) ethylene diamine dihydrochloride (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) followed by reading the absorbance at 538 nm (Spectrophotometer; UV-1100; Shanghai mapada Instruments Co., Ltd, Shanghai, China)[10].

2.5 Microbiological analysis

For microbiological analysis, 10 g of the shredded papaya sample was homogenized in a stomacher (Whirling mixer; QL-861; Haimen Kirin Medical Instrument, Haimen, China) with 90 mL of saline (0.9% NaCl). The homogenates were then serially diluted, and 0.05 mL aliquots of the appropriate dilutions (10^{-1}, 10^{-3}, 10^{-5}) were spread on plates of MRS agar supplemented with 1% $CaCO_3$ to isolate LAB (Superclean bench; SW-CJ-IFD; purification Engineering Equipment Co., Ltd. Suzhou Jiabao, Suzhou, China). All plates were incubated at 30℃ for 48 hrs under anaerobic conditions (Anaerobic box; TE-HER84 Hard Anaerobox, ANX-1; Hirosawa Ltd, Tokyo, Japan). Aerobic bacteria were isolated and counted on Nutrient agar which were incubated at 37℃ for 48 h under aerobic conditions[11]. Mold and yeast were cultured for 48 hrs at 30℃ on Potato Dextrose agar.

2.6 Sensory analysis

Sauerkraut was evaluated after a week. A taste panel of five trained persons evaluated the intensity of attributes of various samples. The sauerkraut samples were evaluated for aroma, taste and texture. The samples were tasted in randomized order as blind tests. Prior to each random test, the mouth was rinsed with water, following the evaluation standards described by Chen[12].

3 Results and discussion

Based on the results of one-variable-at-a-time method, the minimum and maximum limits of the variables were sugar (A) 2%-6%, salt (B) 2%-6%, amount of inoculum 1-5 mL/100 g papaya (C) and temperature 20-40℃ (D). Then central composite design (CCD) of the RSM was used for the final optimization experiment. Central composite design matrix of the variables along with the experimental (n=3) and predicted values of total acid are given in Table 1.

Analysis of variance (ANOVA) is showed in Table 2. The analysis of variance (ANOVA) of the quadratic regression model demonstrated that the model was highly significant (p<0.0001). The model F-value was 42.23 for total acidy. An insignificant value of Lack of Fit (Probability P>F=0.0562) means that the range of variables and levels used in the CCD were compatible. And the goodness of fit of the model was checked by coefficient of determination (R^2). R^2 was 0.98. It can be expressed in percentage also and interpreted as the percent variability in the response in the given model.

As per the model, sample variation of 98.00% for total acidy was attributed to the independent variables. The RSM gave the following regression equations for the total acid (R1) as a function of sugar (A), salt (B), temperature (C), and inoculum (D). The final equation in terms of coded factors is:

$$R1 = +8.77 + 0.29A - 0.59B + 1.37C + 1.61D + 0.17AB - 0.16AC - 0.23AD + 0.57BC - 0.11BD - 0.45CD - 0.32A^2 - 0.53B^2 - 1.9C^2 - 0.34D^2$$

Table 1 Central composite design matrix of the variables along with the actual and predicted values of total acid

No.	Sugar /%	Salt /%	Temperature /℃	Inoculum /mL ·100 g^{-1} papaya	Total acid/g ·kg^{-1} Actual	Total acid/g ·kg^{-1} Predicted
1	6	4	20	3	5.54	5.63
2	4	2	20	3	6.35	6.13
3	4	2	30	5	10.03	10.21
4	4	4	30	3	8.82	8.77
5	2	4	20	3	4.32	4.73
6	6	6	30	3	7.61	7.78
7	4	2	30	1	6.57	6.76
8	2	4	30	5	9.43	9.66
9	4	6	20	3	3.60	3.80
10	2	6	30	3	6.89	6.86
11	4	4	30	3	8.46	8.77
12	4	4	30	3	8.78	8.77
13	4	4	20	1	3.06	3.09
14	2	4	30	1	6.30	5.97
15	4	4	30	3	8.82	8.77
16	4	4	40	5	9.00	9.06
17	4	4	20	5	7.74	7.22
18	4	2	40	3	7.92	7.72
19	4	6	30	1	6.08	5.8
20	4	6	40	3	7.47	7.69
21	6	4	30	1	7.25	7.02
22	6	2	30	3	8.51	8.63
23	4	6	30	5	9.09	8.81
24	4	4	30	3	8.96	8.77
25	6	4	30	5	9.45	9.78
26	6	4	40	3	8.55	8.05
27	2	2	30	3	8.46	8.38
28	2	4	40	3	7.96	7.78
29	4	4	40	1	6.12	6.73

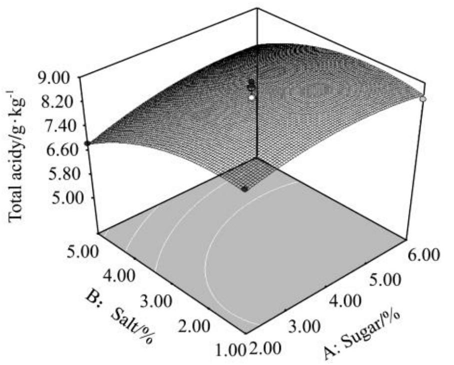
Table 2 Analysis of variance (ANOVA) for the fitted quadratic polynomial model of total acid

Source	SS	DF	MS	F-value	P-value
Model	85.23	14	6.09	42.23	<0.0001**
A	1.04	1	1.04	7.2	0.0178*
B	4.2	1	4.2	29.14	<0.0001**
C	22.47	1	22.47	155.87	<0.0001**
D	31.27	1	31.27	216.91	<0.0001**
AB	0.11	1	0.11	0.79	0.389
AC	0.099	1	0.099	0.69	0.4206
AD	0.21	1	0.21	1.48	0.2433
BC	1.32	1	1.32	9.18	0.009**
BD	0.05	1	0.05	0.34	0.5672
CD	0.81	1	0.81	5.62	0.0327*
A^2	0.67	1	0.67	4.63	0.0494*
B^2	1.84	1	1.84	12.73	0.0031**
C^2	23.4	1	23.4	162.36	<0.0001**
D^2	0.75	1	0.75	5.19	0.039*
Residual	2.02	14	0.14		
Lack of Fit	1.88	10	0.19	5.57	0.0562
Pure Error	0.14	4	0.034		
Cor total	87.24	28			

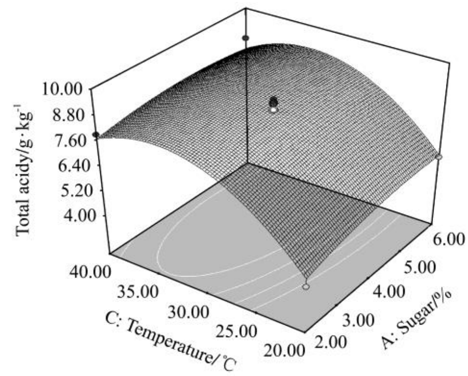
Note: SS, Sum of squares; DF, degree of freedom; MS, mean square, C.V. = 5.07%, R^2 = 0.98.
P≤0.01 was very significant, with the "**" indicates; 0.01<P≤0.05 was significant, with the "*" indicates; P>0.05 was not significant.

The equation and Table 2 suggested that sugar (*A*), salt (*B*), temperature (*C*), and inoculum (*D*) had direct influence on total acid. The interaction coefficients *BC* and *CD* were significant for total acid while others were not significant. The observation suggested that in the case of total acid, temperature exhibited significant interaction with salt and inoculum. This indicated that temperature played an important role in the fermentation of sauerkraut. However, sugar showed insignificant interaction with salt, temperature and inoculum. Effects of interaction of varying concentrations of sugar and salt, sugar and different temperature, sugar and inoculum, salt and varying temperature, salt and inoculum, and inoculum and different temperature on total acidy production were shown when all other parameters at optimum are presented in Figures 1 (a-f). Through the optimization of the response surface, the optimum concentrations of sugar, salt and inoculum we obtained were 3.8%, 2.8% and 5.0 mL/100 g papaya, and optimum temperature was 31.23℃.

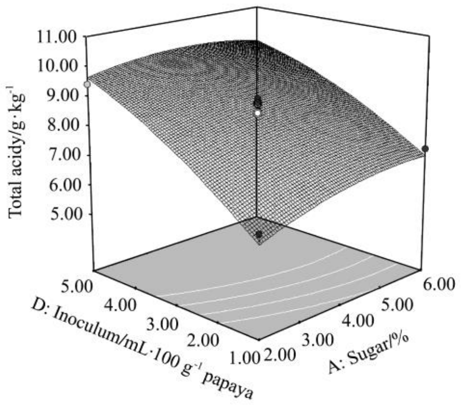
To confirm this result, experimental rechecking was performed. Indicators of the sauerkraut determined after three days were shown in Table 3.



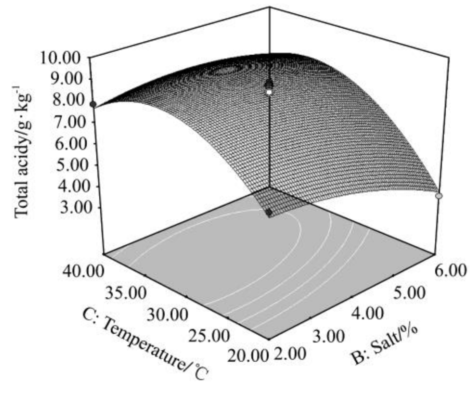
a. Sugar and salt



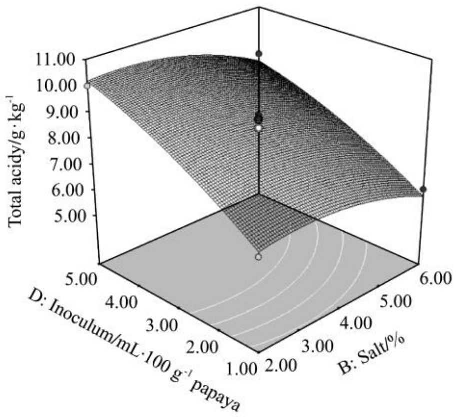
b. Sugar and temperature



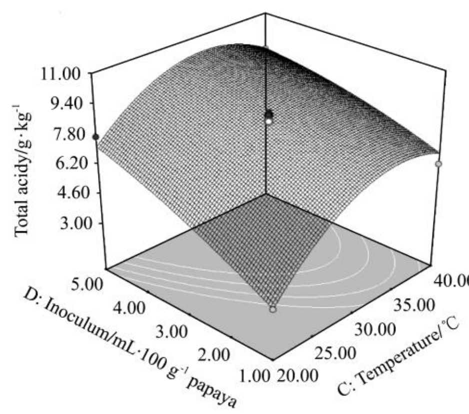
c. Sugar and inoculum



d. Salt and temperature



e. Salt and inoculum



f. Temperature and inoculum

Figure 1 Response surface plots of total acid on sugar, salt, temperature and inoculum

Table 3 Analysis of papaya sauerkraut fermented by optimized process

Source	Total acidy /g kg^{-1}	Total sugar /g kg^{-1}	Nitrite /mg kg^{-1}	LAB /CFU mL^{-1}	Fungus /CFU mL^{-1}	Total bacterial count /CFU mL^{-1}	Score of sensory evaluation
Sauerkraut	9.32	17.63	0.00	6.65×10^{8}	6.34×10^{4}	6.72×10^{7}	90.67

Note: Values are means of three replicates.

4 Conclusions

The study suggested that central composite design of RSM was reliable for optimizing the process of pickle fermentation. The final composition of the optimized process was: 3.5% sugar, 2.3% salt, and 5% inoculum at 31℃. This study indicated that salt, inoculation amount and temperature had a direct impact on the quality of sauerkraut; and the effect of temperature was highly significant; the importance of salt and inoculum amount was next to temperature.

The reason why the immature papaya is chosen as the sauerkraut material is that the sugar content of immature papaya we determined was about 5%-6%, which determines that its poor taste is not suitable for direct consumption and the price is cheap accordingly. The greater hardness of immature papaya makes it a better material for sauerkraut production than ripe papaya. The taste, color and flavor of sauerkraut fermented with immature papaya were better than that with ripe papaya, too. The sugar content of ripe papaya is 10%-14%, which is higher than that of immature papaya. Sold at a high price, ripe papaya is a popular fruit because of its good taste and low hardness. If fully ripe papaya is used to ferment sauerkraut, the sauerkraut would be difficult for molding and its juice would be turbid, which leads to a bad taste. Meanwhile, the cost would be too high.

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