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Application of Compound Preservatives in Beijing Sausages

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Abstract: The purpose of this study was to explore the effect of natural and safe compound preservatives on the shelf life of Beijing sausages. In this thesis, Pseudomonas sp was the first dominant bacterium in the Beijing sausages, which was taken as the research object. The total number of bacteria and the sensory score were taken as the evaluation indexes. On the basis of previous studies, Cinnamon, clove and fennel had good antibacterial effect and those were selected as compound preservatives. By using three-factor quadratic universal rotation design experiment and establishing the mathematical model. Then the effect of single factor experiment shown that within the coding range, the bacteriostatic effects of anise, cinnamon and clove were gradually weakened, the effect of interaction experiment shown that there was significant interaction between cinnamon and clove. When the total number of bacteria reached the lowest level, the optimal ratio of the compound preservatives was determined as follows: cinnamon 0.063 g/mL, clove 1.000 g/mL, fennel 0.810 g/mL. According to this formula, 0.1% of compound preservative was added to the meat and compared to the control group, under the same conditions and time of storage, the total number of bacteria was significantly lower. The result shown that The shelf life of sausages could be extended by 9 days by adding 0.1% compound preservatives.

Keywords: Compound Preservatives, Beijing Sausages, Application

1. Introduction

The Beijing sausage is a kind of low-temperature meat products, which has high quality, delicious taste and strong fragrance. However, in the process of storage, transportation and marketing, due to the increase of microorganisms, it is easy to spoil and decay, and the shelf life of product is often short, which greatly limits the development of such products [1]. Natural spices, as common food condiments and preservatives, are healthy, non-toxic and safe [2-3]. Spices such as anise, clove and cinnamon are common excipients in meat processing [4-6]. They have bacteriostatic, antioxidant and flavoring effects [7-11]. Hernndez found that the inhibitory concentration of cumin and clove volatile oil on five common pathogenic bacteria in meat was 500 mg/L and 750 mg/L, respectively, which had significant effect on meat preservation [12]. Cinnamon extract has obvious bacteriostatic effect on Pseudomonas spp., Cyclosporus thermophilus and Escherichia coli [13]. Benmalek and others extracted flavonols and anthocyanins from fennel. The results show that these substances have good inhibitory effect on Escherichia coli. Their water-soluble volatile components had broad-spectrum antimicrobial activity, and their antimicrobial activity was obviously stronger than the oil phase of fennel volatile components [14-15]. Tian Feng found that anise, cinnamon and clove showed obvious bacteriostatic effect [16]. Zhu Jiankai's beef prepared with natural spices could be stored for more than 18 days, 3-6

days longer than the general storage [17]. The purpose of this article is to explore a natural compound preservative to prolong the shelf life of Beijing sausages.

2. Materials

2.1. Materials

Natural spices, purchased at Beijing Huilong Guanjing Huitang Peking Pharmacy, meat, purchased at Qiliqu Agricultural and Trade Market

2.2. Sample Preparation

The natural spices were removed from impurities. After crushing by crusher, they were sieved by 40 meshes and soaked in solvent for 12 hours. The extraction was carried out by ultrasonic-assisted extraction for 1 hour. Then, the solution was purified and volume-fixed into a volumetric bottle (concentration 1 g/mL), and stored in a refrigerator at 4°C for reserve.

The meat was processed by the Beijing sausages processing technology of PengCheng Food Company.

3. Methods

3.1. Activation of Indicator Bacteria

Pseudomonas sp. frozen with glycerol was restored at normal temperature. It was poured into 50 mL sterilized nutritious broth medium and cultured at 30°C for 12 hours.

3.2. Three-Factor Quadratic General Rotary Test Design

Using three-factor quadratic general rotary design test [18, 19], the selected three preservatives cinnamon, clove and anise were divided into five levels (the highest extracting concentration of spices was 1g/mL and the lowest bacteriostatic concentration was the lowest). Cinnamon, clove and anise were expressed by X_1, X_2 and X_3, respectively. According to the level coded in table 1, the concentrations of cinnamon, clove and anise were the highest. Aseptic distilled water was used to prepare the solution.

Table 1. *Codes of the factor levels.*

Coding value	Cinnamon X_1 (g/mL)	Clove X_2 (g/mL)	Star anise X_3 (g/mL)
Upper arm number +1.682	1.000	1.000	1.000
Upper level +1	0.810	0.800	0.810
Benchmark level 0	0.532	0.508	0.532
Lower level -1	0.253	0.215	0.253
Lower arm number -1.682	0.063	0.016	0.063

By doing bacteriostatic experiments. The total bacterial count (CFU/mL)/10^5 was taken as the evaluation index value Y. According to the three-factor quadratic general rotary design part of DPS agricultural experiment statistical analysis software, the evaluation value Y was input into the computer to get the statistical results. The results were analyzed by multiple regression analysis using the analysis software, and the regression equation was obtained for variance analysis.

3.3. Effect of Compound Preservatives on Beijing Sausages

After obtaining the formula of compound preservative, added it to the sausages and carried out sensory evaluation test on the samples on the first day of production. During the storage period of the samples, the microbial indexes of the samples were analyzed to study the antiseptic effect of composite preservatives on the Beijing sausages.

4. Results and Discussion

4.1. Test Results of Quadratic General Rotary Design with Three Factors

Table 2. *Results of three factors quadratic rotation design.*

Test number	X_1	X_2	X_3	Y
1	1	1	1	21
2	1	1	-1	28
3	1	-1	1	32
4	1	-1	-1	41
5	-1	1	1	30
6	-1	1	-1	49
7	-1	-1	1	84
8	-1	-1	-1	137
9	1.682	0	0	30
10	-1.682	0	0	79
11	0	1.682	0	24
12	0	-1.682	0	93
13	0	0	1.682	69
14	0	0	-1.682	116
15	0	0	0	52
16	0	0	0	56
17	0	0	0	62
18	0	0	0	49
19	0	0	0	44
20	0	0	0	59

4.2. Variance Analysis

The data in Table 2 were analyzed by the statistical analysis software of DPS agricultural experiment. The regression equation was obtained with the total number of bacteria (CFU/mL)/10^5 as dependent variable and the additive concentration of cinnamon, clove and anise as independent variables.

$$\begin{aligned} Y = {} & 54.20199 - 19.06672X1 - 20.65111X2 - 12.23100X3 - 3.21673X12 \\ & -1.80286X22 + 10.21499X32 + 14.75000X1X2 + 7.00000X1X3 + 4.50000X2X3 \end{aligned} \quad (1)$$

Variance analysis of the variables in the regression equation is carried out, and the results are shown in Table 3.

Table 3. *Result of variance analysis.*

Source of variation	Sum of squares	Freedom	mean square	partial correlation	F value	P value
X_1	4965.3173	1	4965.3173	0.8791	34.0164	0.0002
X_2	5824.8112	1	5824.8112	0.8942	39.9046	0.0001
X_3	2043.2371	1	2043.2371	0.7637	13.9978	0.0038
X_1^2	149.1733	1	149.1733	0.3045	1.022	0.3359
X_2^2	46.8584	1	46.8584	0.1764	0.321	0.5835
X_3^2	1504.3153	1	1504.3153	-0.7124	10.3058	0.0093
X_1X_2	1740.5	1	1740.5	-0.7375	11.9238	0.0062
X_1X_3	392	1	392	-0.4601	2.6855	0.1323
X_2X_3	162	1	162	-0.3161	1.1098	0.3169
regression	16980.0659	9	1886.674	F2=12.92522		0.0002
Surplus	1459.6841	10	145.9684			
Loss of imitation	1238.3508	5	247.6702	F1=5.59496		0.051
deviation	221.3333	5	44.2667			
sum	18439.75	19				

The result of variance analysis were shown that the p value of F test corresponding to the regression equation was 0.0002 < 0.05, which reached a very significant level. It showed that the regression model was effective and could reflect the actual situation in the experiment to the maximum extent. The p value of the missing item was equal to 0.051 > 0.05, which showed that the model fit the test well and the test error was small.

And the effects of X_1, X_2 and X_3 on Y value were highly significant, and the absolute values of regression coefficient of quadratic terms of regression equation were $X_3 > X_1 > X_2$ in turn, indicating that among the compound preservatives, anise had the most significant bacteriostatic effect on Pseudomonas, followed by cinnamon and clove. In addition, there was a significant interaction between cinnamon and clove, but there was no significant interaction between cinnamon and anise, clove and anise.

4.3. Analysis Results of Single Factor Effects

Two factors in the regression equation were controlled at zero level, and the single factor effect equation was obtained to analyze the bacteriostatic effect of a single spice on Pseudomonas sp. The three single-factor effect equations are as follows:

$$Y1=54.20199-19.06672X1-3.21673X12 \quad (2)$$

$$Y2=54.20199-20.65111X2-1.80286X22 \quad (3)$$

$$Y3=54.20199-12.23100X3+10.21499X32 \quad (4)$$

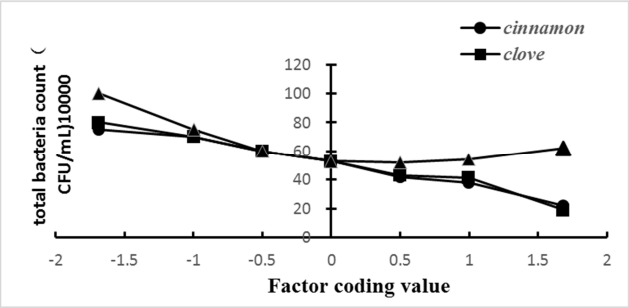

Figure 1. *The curve of single factor effect.*

According to the single factor equation, the single factor effect curve is obtained, as shown in Figure 2. It showed that the bacteriostatic effect increases with the increase of cinnamon and clove dosage, and the bacteriostatic effect of anise increased first and then decreased with the increase of its concentration.

4.4. Marginal Effect Analysis

The first-order partial derivative of the single-factor effect equation is obtained, and the single-factor marginal effect equation is obtained.

$$d\ Y1/d\ X1=-19.06672-6.43346X1 \quad (5)$$

$$d\ Y2/d\ X2=-20.65111-3.60572X2 \quad (6)$$

$$d\ Y3/d\ X3=-12.23100+20.42998X3 \quad (7)$$

The single factor marginal effect equation reflected the change rate of Y value (total number of Pseudomonas colonies) with three different spice coding values (concentration level). According to the single factor marginal effect equation, the single factor marginal effect map is obtained, as shown in Figure 2.

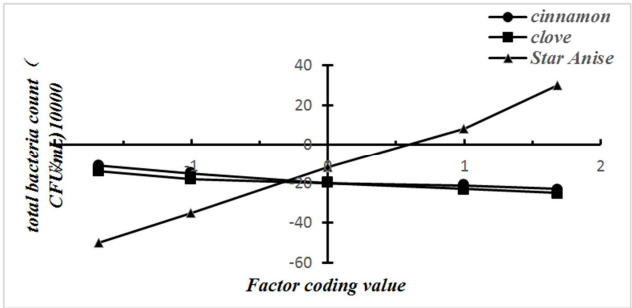

Figure 2. *The curve of single factor boundary effect.*

It showed that the concentration of anise had the greatest influence on the total bacterial count, followed by cinnamon and clove. In addition, at higher concentrations, the anise concentration on the change rate of total bacterial count was increasing.

4.5. Interaction Effect Analysis Results

The regression equation was analyzed by dimensionality reduction. Another factor (anise) was fixed at zero level, and the interaction equation between cinnamon and clove was obtained.

$$Y12 = 54.20199 - 19.06672X1 - 20.65111X2 - 3.21673X12 - 1.80286X22 + 14.75000X1X2 \quad (8)$$

According to the interaction equation, the interaction curves of cinnamon and clove are obtained, as shown in Figure 3.

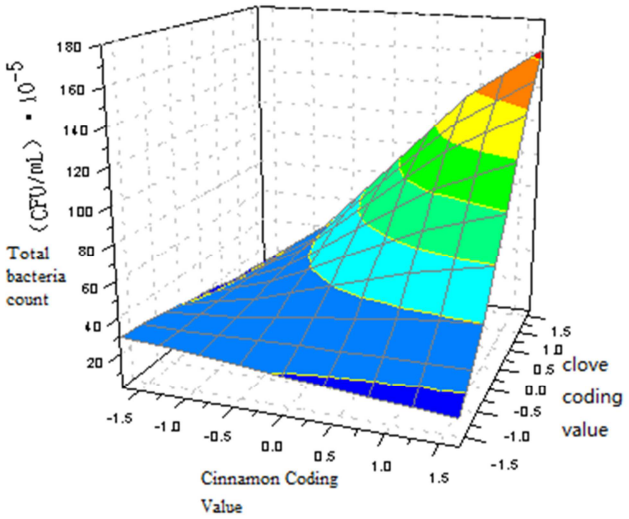

Figure 3. *The curve of interaction between Cinnamomum cassia and Syzygiumaromaticum.*

Figure 3 showd that when cinnamon and clove were added the least, the total bacterial count reached the minimum, and the bacteriostasis effect of compound preservatives was the best.

4.6. Determination of the Optimum Concentration of Composite Preservative

By analyzing the results of three-factor quadratic general rotary design test, it could be concluded that the total number of bacteria (CFU/mL)/10^5 reached the lowest level. When Y = 7.8*10^5 (CFU/mL), the coding values of the three preservatives were as follows: X_1=-1.682, X_2=1.682, X_3=1.000

Converting the coding value to the actual value, the optimum concentration of three preservatives could be obtained as follows: Cinnamon 0.063 g/mL; Clove 1.000 g/mL; Star anise 0.810 g/mL.

4.7. Sensory Evaluation Results

Compared with No.1 sample without compound preservatives, the sample with compound preservatives had no nausea and nausea taste, and has a light flavoring taste, so that the meat belly did not taste greasy.

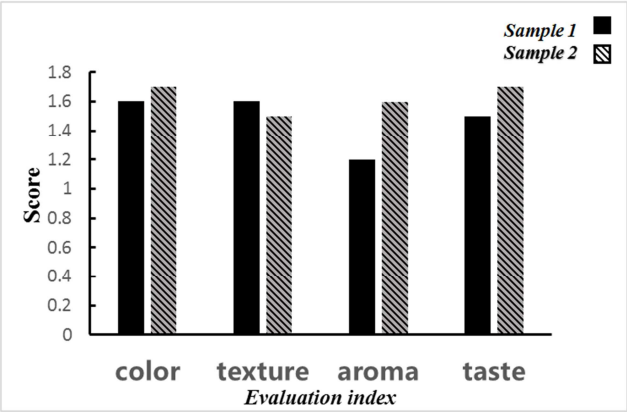

Figure 4. *Sensory evaluation.*

4.8. Determination of Microbial Indicators During Storage

Table 4. *The results of microbial index determination on sample (1).*

	Day 0-6	Day 7	Day 8
total numbers of colony (CFU/g)	<10	62000	75000
Coliform population (MPN/100g)	0	0	130

Table 5. *The results of microbial index determination on sample (2).*

	Day 0-8	Day 9	Day 10	Day 11
total numbers of colony (CFU/g)	<10	7800	55000	98000
Coliform population (MPN/100g))	0	0	300	330

On the 7th and 8th days of storage, the total number of colonies and coliforms exceeded the national standards, respectively. The shelf life of No. 1 sample was 9d. On the 10th day of storage, the total number of colony and coliform bacteria exceeded the requirements of the national standard, so the shelf life of No. 2 pork belly sample was 9 days.

5. Conclusion

In this experiment, the optimum formula of compositive preservatives was obtained by three-factor quadratic general rotary design method: cinnamon 0.063 g/mL, clove 1.000 g/mL, anise 0.810 g/mL. It can extend the shelf life for 9 days when applied to Beijing sausages.

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