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Optimization of the conditions for pepper soaking peeling by enzymatic method

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ABSTRACT

The effect about peeling of fresh pepper by enzymatic soaking method was investigated. A high degree of peeling and good quality from white pepper were demonstrated in a set of well-designed experiments. The conditions for the enzyme-soaking peeling of fresh pepper were optimized by single-factor test and multi-factors orthogonal test. Results showed that the optimal conditions for this process were determined as: pectate lyase to plant hydrolase ratio of 3:2(v/v), concentration of complex enzymatic solution 2.5 % (v/w), the pH value of the supplementary solution about 3.6, and time of 5 min blanching in boiled water. Under these conditions, the rate of peeling was 98.3% after the pepper were soaked for 16 h. The peeled white pepper got rid of the foul smell and had bright and white color. Volatile oil and piperine contents of the product were respectively 2.12 % (v/w) and 4.17 % (w/w).

KEYWORDS

Pepper;
Microbial enzyme;
Soaking peeling;
Conditions.

INTRODUCTION

Pepper (*Piper nigrum* L.), the most popular spice for its pungency and flavour[1], are commercially available as black, white, and fresh pepper. Black pepper, produced from unripe fresh berries of the pepper plant by sun-drying, is known as the ''king of spices''[2]. White pepper, produced from mature fruits by removing the outer layers of the pericarp[3,4], is preferred over black pepper as its mild flavour and charming creamy white color[5].

At present, the commonly used method for pepper peeling is water retting, with the disadvantages of long period and large amount of water consumption. It may result in the bad appearance of the pepper with serious microbial pollution and foul smell, greatly restricting the sustainable development of the domestic pepper planting industry and processing industry[6,7]. The use of microbial enzymes for peeling is an effective method, which mainly applies the enzyme produced by microorganism to degrade the original pectin, pectin and cellulose in the pericarp,

to achieve the goal that softening pericarp and peeling[8]. It had been applied in the degumming of the bast fiber plants and received a wide range of acceptance[9]. In recent years, the preliminary research about the pepper peeling with microbial enzyme has been carried out at home and abroad[7,8,10]. So far, the studies mainly focus on the selection of the microorganism and the analysis of the peeling effect, however, less researches pay attention to the conditions for the commercial enzymatic peeling of pepper.

Here, we propose a new process of soaking peeling about complex enzymatic solution that can peel fresh pepper pericarp in a shorter time and make it to give out flavour without odour. This method achieves a high peeling rate, and it also shortens peeling time and gets rid of odour, compared with the conventional water-retting method. The purpose of the present study is to investigate the peeling effect of enzymatic solution on fresh pepper. A single factor and multi-factor orthogonal tests were applied to optimize the effect of ratio of the complex enzyme, time of boiled water blanching, concentration of the complex enzyme and pH of the buffer solution. Results obtained from orthogonal test optimization study and quality analysis of white pepper are presented.

MATERIALS AND METHODS

Materials

Fresh peppers (*Piper nigrum* L.) were picked from Nanyang state farm in Wenchang, which is located in the east part of Hainan province of China. Commercial grade pectate lyase (activity ≥ 22500 U/mL)and plant hydrolase (activity ≈100 FEB/g) were purchased from Guangzhou Mingyao Trade Co. Ltd., China. Cellulase (with activity ≈ 300 U/mL) and acidic pectinase (with activity ≈ 200 U/mL) were purchased from Shandong Sukahan Bio-technology Co. Ltd.,China. Pectate lyase and plant hydrolase were liquid state, cellulase and pectinase were solid particles. All other chemical reagents were analytical grade made in China.

The process of peeling about pepper

A certain amount of fresh pepper were soaked in water or enzymatic solution for several hours or days under the normal temperature. When pericarps of pepper were softened, pericarps were kneaded by hand and washed with water to remove the pomace. At last, peeled wet peppers were dried under 50 ~ 55 to 12% moisture content.

The rate of peeling(R) about pepper

Dried white pepper of about 100 g were weighed by analytical balance, which signed as sample M_2. White pepper that is completely peeled (not glued leather) were picked out from sample M_2, which signed as sample M_1. Every sample were weighed repeatedly three times and peeling rate (R) are calculated respectively. Average of test results obtained for three repetitive tests were analyzed to evaluate the reproducibility of peeling rate.

$$R(\%) = \frac{M_1}{M_2} \times 100\%$$

R - peeling rate, M_1 - white pepper quality (g) of completely peeling, M_2 (g) - sampling total quality.

The quality evaluation of white pepper

The quality of white pepper produced by enzymatic soaking peeling and water retting was evaluated with respect of color, aroma, volatile oil content, and piperine quantity. Volatile oil content and piperine quantity were separately estimated by the standard methods[7,8]. Aroma of the white pepper were estimated by the sensory evaluation. Color of the white pepper were measured by colorimeter. The light value measurement was according to CIELAB system. L= 0 means black, and L=100 means bright. $L \geq 4$, colour and lustre is normal; $2.5 \leq L < 4$, colour and lustre is a bit dark, acceptable; $L < 2.5$, dark color, unacceptable.

Selection of enzyme preparations.

5 kg fresh peppers were soaked in 4.5 L pH 4.0 buffer solution (disodium hydrogen phosphate-citric acid),a total of five. Then, 5% (v/w) pectate lyase, 5% (v/w) plant hydrolase, 0.5% (w/w) cellulase, 0.5% (w/w) acidic pectinase and control group (no any anzyme) were added respectively for soaking peeling. The peeling rate was calculated

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every 12 h, totally 60 h. Concentration (v/w) of pectate lyase and plant hydrolase was prepared according to volume of the enzymatic solution per kilogram of pepper. Concentration (w/w) of cellulase and acidic pectinase were calculated according to weight of the enzymatic solution per kilogram of pepper. The pericarp softness was observed and the R of soaked pepper was calculated every 12 h, totally 60 h.

Ratio determination of the complex enzyme

5 kg fresh pepper were soaked in 4.5 L buffer solution (pH 4.0) respectively, seven in total. Add 5 % complex enzymatic solution with the ratio of pectate lyase to plant hydrolase respectively as follows: 0:5, 1:4, 2:3, 1:1, 3:2, 4:1 and 5:0 (v/v). The pericarp softness was observed and the R of soaked pepper was calculated every 12 h, totally 48 h.

Time determination of blanching time in boiled water

Plenty of fresh pepper were blanched by boiled water for 0 min,1 min,3 min, 5 min, 7 min, 8 min and 9 min. 1 kg blanched pepper were soaked in 0.9 L disodium hydrogen phosphate-citric acid buffer solution (pH 4.0) respectively, seven in total. 5% complex enzymatic solution(3:2) were added for soaking peeling for 24 h.

Concentration determination of the complex enzymatic solution

5 kg fresh pepper which blanched 5 min with boiled water were soaked in 4.5 L buffer solution (pH 4.0) respectively, six in total. The concentration (v/w) of complex enzymatic solution added respectively was 0, 1.25%, 2.5%, 5%, 7.5% and 10%. The rate of peeling was calculated after blanched fresh pepper were soaked for 24 h.

Determination the best pH value of the buffer solution

Different pH value buffer solution were prepared as follows: 2.4, 2.8, 3.2, 3.6, 4.0, 4.4 and 4.8. 1kg fresh pepper which blanched 5 min in boiled water were soaked respectively in 0.9 L different buffer solution, six in total. Take the distilled water as the control group. 2.5% complex enzymatic solution (3:2) was added for soaking peeling. The rate of peeling was calculated after 24 h.

Determination of the soaking time

Different soaking time were as follows : 0, 4, 8, 12, 16, 20 and 24 h. 1 kg fresh pepper which blanched 5 min in boiled water were soaked respectively in 0.9 L buffer solution that pH value of buffer solution was 3.6. 2.5% complex enzymatic solution(3:2) was added for soaking peeling. The rate of peeling was calculated after blanched fresh peppers were soaked for corresponding time.

Multi-factors orthogonal test

In the condition of buffer solutions which pH value was 3.6 under the normal temperature, three levels and four factors were designed as an orthogonal experiment of $L_9(3^4)$ form.

The data analysis

All figure were drawn according to the origin 6.0 and all data were analyzed according to Spss 17.0

RESULTS AND DISCUSSION

Selection of enzyme preparations on pepper peeling

TABLE 2 presented the results of selection of enzyme preparations. Among the enzyme used for fresh pepper peeling, pectate lyase was found to be the most effective. Soaking-peeling processes with different enzymatic solution for 60 h, the best R value

TABLE 1 : The design table of factors and levers $L_9(3^4)$

Level	A the concentration of complex enzyme solution(%)	B Soaking time (h)	C the ratio of pectate lyase to plant hydrolase	D blanching time (min)
1	2.00	8	2:3	3
2	2.50	12	1:1	5
3	3.00	16	3:2	7

TABLE 2 : Influences of different enzyme liquids on pepper peeling

Time (h)	(R) % (A)	(R) % (B)	(R) % (C)	(R) % (D)	(R) % (E)
12	0	0	0	0	0
24	20.8±0.5	0	0	0	0
36	43.1±0.4	21.3±0.5	0	0	0
48	79.6±0.6	44.6±0.6	32.7±0.4	20.4±0.5	0
60	90.3±0.5	60.4±0.6	50.1±0.6	47.2±0.4	6.3±0.5

A: pectate lyase; B: plant hydrolase; C: cellulase; D: pectinase; E: control group.

TABLE 3 : Influence of the ratio of the complex enzyme on pepper peeling

Time (h)	Peeling ratio (R) %*						
	A	B	C	D	E	F	G
12	0	0	0	0	0	0	0
24	0	19.6±0.5	20.3±0.5	20.1±0.5	20.8±0.4	18.5±0.4	20.8±0.5
36	21.3±0.4	37.3±0.4	62.9±0.5	76.1±0.7	79.2±0.6	42.1±0.5	43.1±0.4
48	44.6±0.4	75.9±0.6	89.3±0.7	91.4±0.6	93.8±0.5	79.2±0.5	79.6±0.6

Ratio of pectate lyase to plant hydrolase respectively was 0:5 (A), 1:4 (B), 2:3 (C), 1:1 (D), 3:2 (E), 4:1 (F) and 5:0 (G).

could reach 90.3%, achieved by the pectate lyase. The second was the plant hydrolase solution in which R was 60.4%. The worst effect was caused by the control group in which R was 6.3%. Therefore, the pectate lyase and plant hydrolase were chosen to be used in a series of experiments. However, K.M.Gopinathan have reported that pectinase was the key enzyme for pepper peeling[7]. The mechanism about pectinase, pectate lyase and plant hydrolase that softened pepper pericarp is still unclear.

The peeling effect about the ratio of the different complex enzymatic solution

The result of peeling with different ratios of pectate lyase and plant hydrolase was shown as TABLE 3. The soaking effect of the complex enzyme was significantly better than that of the single enzyme. When the ratio of the complex enzyme was 3:2 (v/v), the percicap can be completely peeled after soaking for 48 h. So in the following experiments, the best effective ratio of the complex enzyme was 3:2 (v/v).

Peeling effects under different time of blanching in boiled water

The results of peeling was shown as Figure 1. Results showed that blanching treatment could accelerate the pepper pericarp to soften. The longer time of blanching was, the better peeling rate it would be. However, Blanching time of more than 8 min could have an adverse effect on the colour and lustre of white pepper. Blanching time of more than 3 min, the rate of peeling were above 95%, but the best colour of white pepper was blanching time of 5 min. Considering peeling rate and colour, the blanching time in boiled water was found to be 5 min (Figure 1).

Peeling effects under different concentration of the complex enzymatic solution

The concentration of the complex enzyme will directly affect the effect of peeling, the higher the concentration of the complex enzyme liquid was, the better the peeling effect it would be (Figure2). After 24 h of soaking, the rate of peeling were 89.3 % and

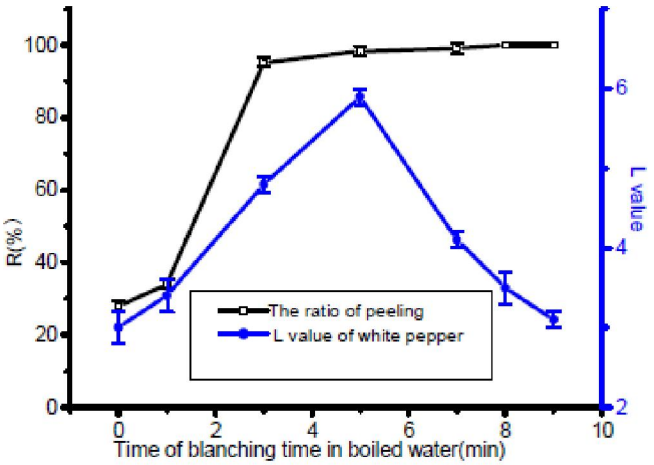

Figure 1 : Peeling effects under different time of boiled water blanching

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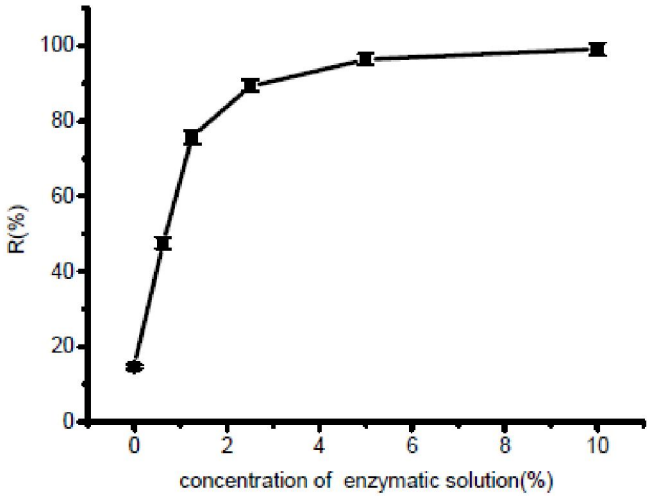

Figure 2 : Peeling effects under different concentration of the complex enzyme

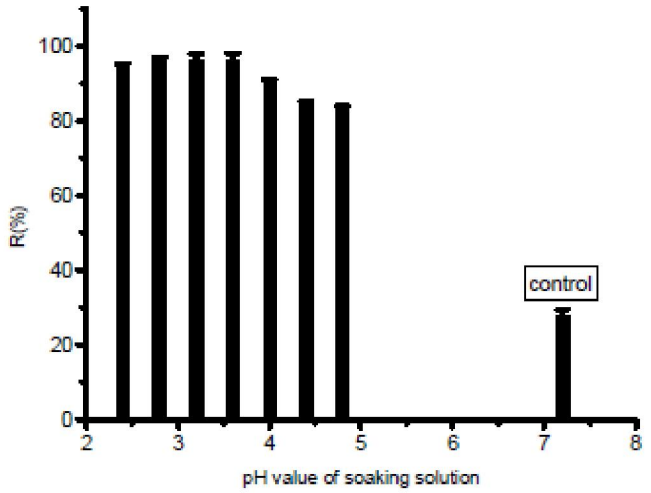

Figure 3 : Peeling effects under different pH values

96.4 % respectively under the condition of the concentration of 2.5 % and 5 %. Considering peeling rate and economic costs of enzyme liquid, the concentration of the enzyme liquid was 2.5 %.

Peeling effects under different pH values

From Figure 3, the complex enzyme had relatively strong activity under the acidic conditions. When the pH value was 4.8, the peeling ratio was 80%. The highest peeling ratio was appeared at the pH value of 3.6. So the best pH value of the supplementary solutions for peeling was 3.6.

Peeling effects under different soaking time

The longer the soaking time of the complex enzyme liquid was, the better the peeling effect it would be (Figure 4). When the soaking time was over 12 h,

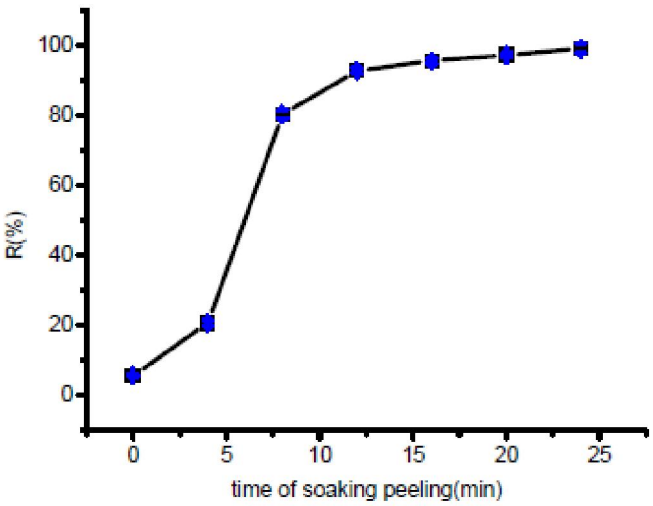

Figure 4 : Peeling effects under different soaking time

the peeling rate was 92.7% and effective. When the soaking time was above 12 h, the peeling rate would increased slowly. So the appropriate time of the soaking-peeling was 12 h.

Conditions optimization for pepper soaking peeling

To determine the optimal conditions for pepper soaking-peeling by enzymatic method, a multi-factor orthogonal test was implemented. In the TABLE 4, Kj_m is sum of the test results of factors in the j column (j = A, B,C,D) and level m (m = 1, 2, 3); X_{jm} is the mean of K_{jm} ($X_{jm} = K_{jm}/3$) and $R_j = (X_{jm})\max$ " $(X_{jm})\min$. The values of R_j were consistent with the effects of factors on various levels. A higher R_j value indicates that greater effect was obtained in the j factor. Results are presented in TABLE 4. Variance analysis results of orthogonal test was showed in TABLE 5.

The significance values of different conditions were in the following order: B > D > C > A. Soaking time was the most critical factor, the second was blanching time, the third was the ratio of pectate lyase to plant hydrolase, concentrationof complex enzymatic solution was the smallest influence factor, the optimum level was $A_3B_3C_3D_2$. The concentration of the enzyme liquid directly affected the cost of peeling, the higher concentration of the enzyme liquid, peeling cost is higher, Low concentration of enzyme solution is favorable for practical production. Three groups of experiments about $A_1B_3C_3D_2$,

TABLE 4 : Orthogonal array design for the optimization of pepper peeling

$L_9(3^4)$	A	B	C	D	R(%)
1	1	1	1	1	82.4 ±0. 7
2	1	2	2	2	91.8 ±0. 6
3	1	3	3	3	96.9 ±0. 6
4	2	1	2	3	86.9 ±0. 5
5	2	2	3	1	92.4 ±0. 7
6	2	3	1	2	97.2 ±0. 6
7	3	1	3	2	89.4 ±0. 6
8	3	2	1	3	93.6 ±0. 4
9	3	3	2	1	92.4 ±0. 7
K_{j1}	271.40	259.20	273.80	266.50	
K_{j2}	275.80	277.40	271.10	278.90	
K_{j3}	275.90	286.50	278.20	277.70	
R_j	4.50	27.30	7.10	12.40	
	A_3	B_3	C_3	D_2	

TABLE 5 : Results of variance analysis about orthogonal test

Sources of variance	Type III sum of squares	df	The mean square	F	Sig.
Correction model	515.836[a]	8	64.479	82.494	.000
intercept	223636.091	1	223636.091	286115.880	.000
concentrition	11.474	2	5.737	7.340	.005
soakingtime	371.401	2	185.700	237.582	.000
ratio	21.608	2	10.804	13.822	.000
blanchingtime	89.880	2	44.940	57.495	.000
error	14.069	18	.782		
total	226379.400	27			
a\total of Correction	529.905	26			

R square = 0.973（adjust R square = 0.962）

$A_2B_3C_3D_2$ and $A_3B_3C_3D_2$ were designed to compare the peeling rate, the results showed the peeling rate was 98.3%, 98.6% and 98.6% in turn. Though the peeling rate of $A_2B_3C_3D_2$ and $A_3B_3C_3D_2$ were higher than the peeling rate of $A_1B_3C_3D_2$, the peeling rate of $A_1B_3C_3D_2$ was the choice of priority because of low cost and high efficiency. So the optimum conditions comprised the following: Concentration of the complex enzyme 2%, ratio of the complex enzyme 3:2, soaking time 16 h, and blanching 5 min in boilded water. Under such conditions for the 16 h enzyme-soaking peeling of pepper, the peeling ratio was 98.3%. These were significantly higher values than those obtained prior to optimization.

Analysis of white pepper quality

Figure 5 and TABLE 6 showed the comparison of the quality of the white pepper produced by the enzyme-soaking peeling and water retting. Results showed from TABLE 6 that the peeling rate, L value,volatile oil and piperine contents of white pepper I were 95.1%,4.08, 2.09 % (v/w) and 4.13% (w/w), of white pepper II were 98.3%, 5.30, 2.12 (v/w) and 4.17% (w/w). There was no evident difference between volatile oil and piperine contents. As for the peeling rate and L value, there are evident differences between them. Soaking way in complex enzymatic solution had the advantage of short peeling time and white colour than traditional soak-

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Figure 5 : (I) white pepper peeled by tranditional water retting; (II) White pepper peeled by the enzyme-soaking

TABLE 6 : Comparation on quality of white pepper by two kinds of peeling methods

White pepper	The time of peeling	R (%)	L value	flavour	Piperine g/100g	Volatile oil (%)
I	10days	95.1	4.08	mild flavour with unpleasant odor	4.13%	2.09
II	16hours	98.3	5.30	mild flavour	4.17%	2.12

I- White pepper peeled by tranditional water retting ; II - White pepper peeled by the enzyme-soaking

ing way in water.

CONCLUSIONS

The liquid of pectate lyase and plant hydrolase could effectively soften the peel of pepper. When the pH value of the supplementary solution was 3.6 in the normal temperature,the complex ratio was 3:2 (v:v), the concentration of enzymatic solution was 2% (v/w), and the blanching time in boiled water was 5 min, blanched fresh pepper were soaked for 16 h,the peeling rate of fresh pepper was 98.3 %. The quality evaluation showed that the obtained white pepper product had got rid of the foul smell, and also had bright and white color, as well as aromatic flavor. Volatile oil and piperine contents of the product were respectively 2.12 % (v/w) and 4.17% (w/w).

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