Study on Compound Stabilizer Formulation for Mango Juice with Skin

Ziyi YANG, Yurong WANG*, Licheng LIU, Zijing WANG, Jiamei LIU

College of Biomedicine and Chemical Engineering, Liaoning Institute of Science and Technology, Benxi 117004, China

Abstract [**Objectives**] The effects of stabilizers such as sodium carboxymethyl cellulose, pectin, sodium alginate and xanthan gum on the stability of mango juice with skin were discussed using mango as the raw material. [**Methods**] The formula and stability of mango beverage with skin were studied using mango juice with skin as the raw material. Four stabilizers, including pectin, sodium alginate and sodium carboxymethyl cellulose, were added to improve the stability of the juice. Based on single factor experiments and a response surface experiment, the stability model of mango juice was established to determine the best compound stabilizer. [**Results**] According to an orthogonal experiment on the stability of mango juice with skin, the optimum technical parameters were as follows: sodium carboxymethyl cellulose 0.20%, xanthan gum 0.08%, sodium alginate 0.18%, and pectin 0.14%, with which the optimal suspension stability was 69.12%. The optimum technical parameters from the response surface experiment were as follows: sodium carboxymethyl cellulose 0.199%, pectin 0.118 5%, sodium alginate 0.163%, and xanthan gum 0.077%, with which the suspension stability was 70.32%. It was found that the stability of mango juice with skin obtained by the response surface experiment was better than that by the orthogonal experiment, and the order of factors affecting the stability of mango was sodium alginate (C) > sodium carboxymethyl cellulose (A) > pectin (D) > xanthan gum (B). [**Conclusions**] The formula of compound stabilizer for mango juice was optimized by the orthogonal experiment and the response surface method, providing a theoretical basis for the actual production of mango juice with skin.

Key words Mango; Stability of fruit juice; Response surface experiment; Optimization

DOI: 10.19759/j.cnki.2164 – 4993.2024.01.019

Mango trees belong to *Mangifera* in Anacardiaceae, and the fruit is yellow, sweet and hardcore when it matures[1]. Mangoes are rich in carotenoids, which can be converted into vitamin A, which is a powerful antioxidant, preventing diseases and cancer[2]. Mangoes are also rich in vitamin E, which can strengthen the immune system. Mangoes contain mangiferonic acid and other compounds, which have anticancer pharmacological effects. Mangiferin has a certain therapeutic effect on diabetic nephropathy[3]. Mangoes have the effects of relieving cough and reducing phlegm, and auxiliary treatment effects against asthma and other diseases[4].

At present, the production of mango juice is mainly based on peeling and juicing. Mango peel is the waste of mango edible processing, and a large number of peel resources are not fully utilized in practice. However, mango peel accounts for 7% – 24% of all fruit[5]. Mango peel is rich in rutin, protocatechuic acid, myricetin, catechin, geniposide and chlorogenic acid[6–7]. Liu *et al.*[8] studied the polyphenols in mango peel by microwave-assisted organic solvent extraction, and found that the extraction rate of polyphenols reached (6.24 ± 0.02)%. Zhao *et al.*[9] extracted mango pomace polysaccharides by a hot water extraction method, and the extraction rate was 9.29%, and it was found that mango pomace polysaccharides had strong antioxidant capacity. Liu *et al.*[10] studied the content of soluble dietary fiber in mango peel and found that the yield was 18.30% under certain conditions. Huang *et al.*[11] found that the yield of mangiferin in mango peel was 4.622 mg/g, and mangiferin had an antibacterial effect.

The stability of fruit juice beverages affects the appearance and taste and the shelf life of beverages. Studies have shown that main factors affecting the stability of fruit juice are intermolecular interaction and particle polymerization in fruit juice, mainly involving pectin, polyphenols, protein, polysaccharides, starch and metal ions, or the result of the interaction between several factors[12]. Colloidal stabilizers increase the viscosity of fruit juice through hydrophilic groups, change the electrical properties of some suspended particles, and finally increase the suspension stability of fruit juice[13]. However, the addition of a single hydrophilic colloid has limited stabilizing effect on the products, and layering occurs in the later stage, so it is necessary to select several stabilizers to compound them, study their suspension stability, centrifugal stability and sensory stability, and solve the problems of pulp flocculation, sedimentation or fading and sticky taste. In this paper, the effects of stabilizers such as sodium carboxymethyl cellulose, sodium alginate, agar and xanthan gum on mango juice with skin were investigated, and the formula of compound stabilizer for mango juice was optimized by an orthogonal experiment and the response surface method, providing a theoretical basis for the actual production of mango juice with skin.

Materials and Methods

Materials and Instruments

Materials and reagents Test materials: Fresh mangoes (purchased from supermarkets).

Experimental reagents: Pectin, sodium alginate, sodium

Received: October 22, 2023 Accepted: December 26, 2023

Supported by Doctoral Research Start-up Fund of Department of Science & Technology of Liaoning Province (2021-BS-250); Doctoral Research Start-up Fund of Liaoning Institute of Science and Technology (2307B16); College Students' Innovation and Entrepreneurship Training Program of Liaoning Institute of Science and Technology (202311430048).

Ziyi YANG (2002 –), female, P. R. China, major: biotechnology.

* Corresponding author. Yurong WANG (1989 –), female, P. R. China, master, devoted to research about exploitation and utilization of biological resources. E-mail: qimuge1989@163.com.

carboxymethyl cellulose, xanthan gum and citric acid (all of which were of food grade, and produced by Shandong Xiwang Sugar Industry Co., Ltd.); white sugar (food grade).

Instruments and equipment FZ-06 juicer, Wenling Baile Crushing Equipment Factory, Zhejiang; digital constant-temperature water bath pot (HH-6), Changzhou Guohua Electric Appliance Co., Ltd.; ultraviolet spectrophotometer (TU-1900), Beijing Purkinje General Instrument Co., Ltd.; GL-21M high-speed freezing centrifuge, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.

Experimental methods

Mango juice making Fresh disease-free mangoes (90% ripe) were weighed and washed, blanched, and cut into pieces with skin. Water was added according a ratio of pulp : water = 1 : 2, and the mixture was uniformly beaten with a beater until there was no obvious mango residue. Filtration was performed with gauze, and 0.09% sucrose and 9% citric acid were added. Finally, the obtained mango juice was diluted to a concentration of 80% for later use.

Determination of suspension stability of fruit juice According to the method of Qin[14], 10 ml of mango juice was centrifuged at 5 000 r/min for 10 min. The absorbance A_0 of the juice before centrifugation and the absorbance A_1 of the supernatant after centrifugation were determined at 660 nm, respectively. The suspension stability (R) of the juice was calculated with distilled water as a blank.

Suspension stability of fruit juice $R\ (\%) = A_1/A_0 \times 100$

Design of single factor experiments The beaten and diluted mango juice was weighed and added into a centrifuge tube. At room temperature, sodium carboxymethyl cellulose (0, 0.04%, 0.08%, 0.12%, 0.16%, 0.20%, 0.24%, 0.28%, 0.32%, 0.36%), pectin (0, 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16%, 0.18%, 0.20%), sodium alginate (0, 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16%, 0.18%, 0.20%), xanthan gum (0, 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16%, 0.18%, 0.20%) and other stabilizers were added in the centrifuge tube, respectively. Their suspension stability was calculated.

Design of orthogonal optimization experiments According to the results of single-factor experiments on pectin, sodium alginate, sodium carboxymethyl cellulose and xanthan gum, four factors, namely pectin, sodium alginate, sodium carboxymethyl cellulose and xanthan gum were selected to design an orthogonal experiment according to the $L_9(3^4)$ orthogonal table. The orthogonal experimental design is shown in Table 1.

Box-Behnken experiment design by response surface methodology According to the Box-Behnken experiment design, combined with the results of single-factor experiments, four factors that significantly affect the suspension stability of mango juice with skin, namely pectin, sodium alginate, carboxymethyl cellulose sodium, and xanthan gum, were selected. Based on single-factor experiments, a response surface analysis method with four factors and three levels was used to optimize the compound stabilizer formula, with the suspension stability of mango juice with skin as the response value. The design factors and parameters of the response surface experiment are shown in Table 2.

Table 1 Orthogonal experiment design table %

	A Sodium carboxymethyl cellulose	B Xanthan gum	C Sodium alginate	D Pectin
1	0.16	0.06	0.14	0.10
2	0.20	0.08	0.16	0.12
3	0.24	0.10	0.18	0.14

Table 2 Factors and levels of Box-Behnken experiments %

Experimental factor	Factor level	Experimental factor	Factor level
	−1	0	1
A Sodium carboxymethyl cellulose	0.16	0.20	0.24
B Xanthan gum	0.06	0.08	0.10
C Sodium alginate	0.14	0.16	0.18
D Pectin	0.10	0.12	0.14

Results and Discussion

Single-factor experiments and results

Effects of carboxymethyl cellulose sodium on the stability of mango juice with skin Carboxymethyl cellulose sodium is a high molecular weight anionic polymer[15], with good water solubility and strong water-retaining property. It combines with substances that are prone to precipitation in fruit juice, reduces the occurrence of flocculent precipitation in fruit juice storage, improves the stability of fruit juice[16], and has good heat and acid resistance. As shown in Fig. 1, the stability of mango juice with skin first increased and then tended to be stabilized with the addition of sodium carboxymethyl cellulose increasing. When the addition amount was 0.20%, the suspension stability of mango juice reached its maximum value, which was 44.0%. When the addition amount exceeded 0.20%, the stability remained almost unchanged. Therefore, it is advisable to choose a sodium carboxymethyl cellulose addition of 0.20%.

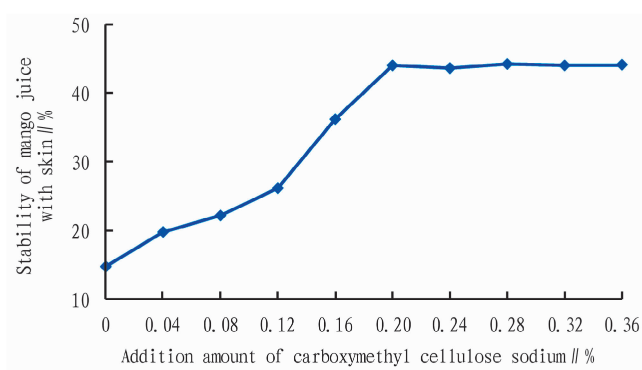

Fig. 1 Effects of carboxymethyl cellulose sodium on the stability of mango juice with skin

Effects of xanthan gum on the stability of mango juice with skin Xanthan gum is a high molecular acidic heteropolysaccharide with high suspension stability. From Fig. 2, it can be seen that the stability of mango juice with skin increased first and then tended to be stabilized with the increase of xanthan gum content. When the addition amount was 0.08%, the stability of mango juice reached its maximum value, which was 43.9%. Therefore, it is advisable to choose an addition amount of 0.08% for xanthan gum.

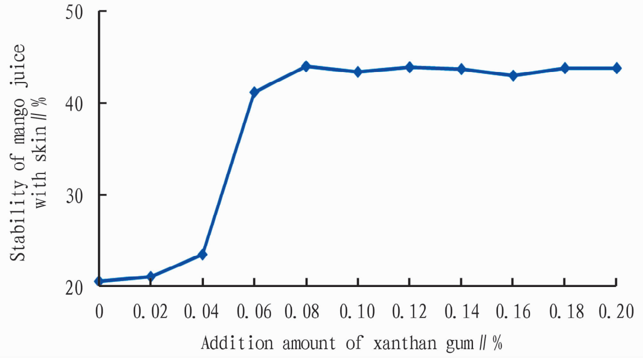

Fig. 2 Effects of xanthan gum on the stability of mango juice with skin

Effects of sodium alginate on the stability of mango juice with skin Sodium alginate is an anionic hydrophilic polysaccharide that is generally extracted from various algae plants[17]. As shown in Fig. 3, the stability of mango juice with skin first increased and then tended to be stabilized with the increase of sodium alginate content. When the addition amount was 0.16%, the stability of mango juice reached its maximum value, which was 44.7%. When the addition amount exceeded 0.16%, the stability of mango juice remained almost unchanged. From an economic perspective, it is advisable to choose an addition amount of 0.16% for sodium alginate.

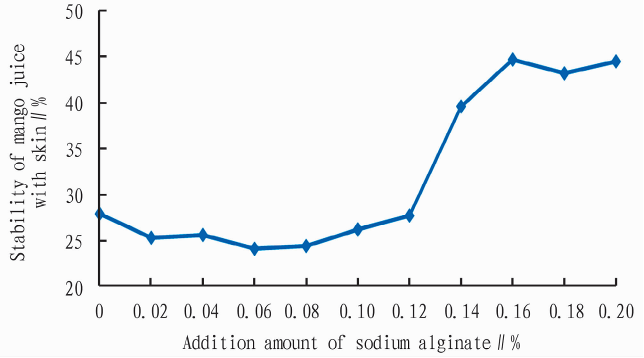

Fig. 3 Effects of sodium alginate on the stability of mango juice with skin

Effects of pectin on the stability of mango juice with skin From Fig. 4, it can be seen that the stability of mango juice with skin increased first and then tended to be stabilized with the increase of pectin content. When the addition amount was 0.12%, the stability of mango juice reached its maximum value, which was 34.8%. Compared with other stabilizers, the effects of pectin on the stability of mango juice was relatively small. It might be because pectin is a negatively charged polysaccharide with low viscosity and limited thickening effect on juice. When the addition amount exceeded 0.12%, the stability remained almost unchanged, and it is advisable to choose a pectin addition amount of 0.12% for mango juice.

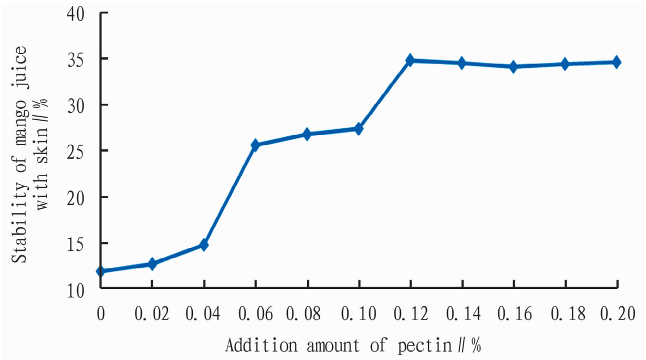

Fig. 4 Effects of pectin on the stability of mango juice with skin

Results and analysis of orthogonal experiments

In order to obtain optimal process conditions for the stability of mango juice with skin treated by stabilizers, an orthogonal experiment was conducted using the stability of mango juice as an indicator. The results are shown in Table 3.

Table 3 Orthogonal experiment results and range analysis table

Factor	Carboxymethyl cellulose sodium A	Xanthan gum B	Sodium alginate C	Pectin D	Stability %
1	0.16%	0.06%	0.14%	0.10%	54.6
2	0.16%	0.08%	0.16%	0.12%	61.5
3	0.16%	0.10%	0.18%	0.14%	68.3
4	0.20%	0.06%	0.16%	0.14%	66.0
5	0.20%	0.08%	0.18%	0.10%	69.1
6	0.20%	0.10%	0.14%	0.12%	53.3
7	0.24%	0.06%	0.18%	0.12%	53.6
8	0.24%	0.08%	0.14%	0.14%	52.9
9	0.24%	0.10%	0.16%	0.10%	52.9
K_1	184.4	174.2	160.8	176.6	
K_2	188.4	183.5	180.4	168.4	
K_3	159.4	174.5	191	187.2	
k_1	61.4	58.0	53.6	58.9	
k_2	62.8	61.2	60.1	56.1	
k_3	53.1	58.1	63.7	62.4	
R	9.7	3.2	10.1	6.3	
Factor ranking	C > A > D > B				
Priority level	A_2	B_2	C_3	D_3	
Optimal combination	$A_2B_2C_3D_3$				

According to the results of orthogonal experiment in Table 3, the main order of the four factors affecting the stability of mango juice with skin was sodium alginate (C) > sodium carboxymethyl cellulose (A) > pectin (D) > xanthan gum (B), that is, the change of sodium alginate had the greatest effect on the stability of mango juice with skin, followed by sodium carboxymethyl cellulose and pectin, and xanthan gum had the lowest effect. The optimum level combination was sodium carboxymethyl cellulose 0.20%, sodium alginate 0.18%, xanthan gum 0.08% and pectin 0.14%. With the above four optimal conditions, its stability was 69.12%.

Results of response surface experiment

Establishment and analysis of regression model In order to optimize the optimum technical conditions for stabilizing mango juice with skin, a four-factor three-level response surface analysis experiment was carried out with sodium carboxymethyl cellulose (A), xanthan gum (B), sodium alginate (C) and pectin (D) as independent variables and the stability of mango juice with skin as the response value. The experimental design scheme and results are shown in Table 4. Totally, 29 experiments were carried out to estimate the experimental error, and the analysis of variance is shown in Table 5. Through the quadratic response surface regression analysis of the experimental results, the regression equation was obtained as follows:

$$R(\%) = 0.74 - 0.035A - 0.059B + 0.033C - 0.011D - 0.17AB + 0.017AC + 0.033AD - 0.055BC + 0.054BD + 0.016CD - 0.19A^2 - 0.21B^2 - 0.14C^2 - 0.12D^2$$

The regression equation was analyzed by variance, and the analysis results are shown in Table 5. As can be seen from Table 5, the model selected in this study was significant ($P = 0.0473$), and the lack of fit was equal to $0.1993 > 0.05$, which was not significant, indicating that the model was reasonable. The correction coefficient R^2 of the model was 0.716 0, which showed that the model had a good fitting degree, and the predicted values had a good correlation with the actual values, and the experimental error was small. Therefore, the model could be used to analyze and predict the stability of mango juice with skin.

According to the analysis of variance, the interaction term AB and quadratic term C^2 had significant effects on the stability of mango juice with skin, while quadratic terms A^2 and B^2 had extremely significant effects. The order of factors affecting the stability of mango juice with skin was sodium alginate (C) > sodium carboxymethyl cellulose (A) > pectin (D) > xanthan gum (B), which was consistent with the results of orthogonal experiments.

Table 4 Design and results of Box-Behnken experiments

No.	Carboxymethyl cellulose sodium A	Xanthan gum B	Sodium alginate C	Pectin D	Stability//%
1	-1	0	1	0	56.5
2	-1	0	-1	0	41.9
3	1	0	1	0	48.9
4	1	0	-1	0	27.5
5	0	0	0	0	58.9
6	1	0	0	1	20.0
7	0	-1	0	-1	58.2
8	0	-1	1	0	41.5
9	0	0	0	0	83.2
10	0	-1	-1	0	24.9
11	0	1	-1	0	28.2
12	1	1	0	0	15.6
13	0	-1	0	1	41.5
14	0	0	0	0	69.1
15	1	-1	0	0	66.1
16	-1	1	0	0	47.8
17	0	1	0	-1	36.3
18	1	0	0	-1	35.8
19	-1	0	0	-1	54.1
20	0	0	1	1	63.8
21	-1	0	0	1	25.2
22	-1	-1	0	0	30.1
23	0	0	-1	-1	46.2
24	0	0	0	0	80.8
25	0	0	-1	1	64.6
26	0	1	1	0	22.7
27	0	0	1	-1	38.9
28	0	1	0	1	41.1
29	0	0	0	0	77.3

Table 5 Variance analysis on results of response surface experiments

Source of variation	SS	*df*	SM	*F* value	*P*	Significance
Model	0.711 3	14	0.050 8	2.521 6	0.047 3	*
A-carboxymethyl cellulose sodium	0.014 4	1	0.014 4	0.719 1	0.410 7	
B-xanthan gum	0.041 5	1	0.041 5	2.061 3	0.173 0	
C-sodium alginate	0.012 6	1	0.012 6	0.629 0	0.440 9	
D-pectin	0.001 4	1	0.001 4	0.073 1	0.790 7	
AB	0.116 2	1	0.116 2	5.770 6	0.030 7	*
AC	0.001 1	1	0.001 1	0.057 3	0.814 2	
AD	0.004 2	1	0.004 2	0.212 9	0.651 6	
BC	0.012 2	1	0.012 2	0.605 9	0.449 3	
BD	0.011 5	1	0.011 5	0.573 4	0.461 4	
CD	0.001 0	1	0.001 0	0.052 4	0.822 2	
A^2	0.233 3	1	0.233 3	11.580 9	0.004 3	**
B^2	0.283 4	1	0.283 4	14.067 7	0.002 2	**
C^2	0.135 5	1	0.135 5	6.726 0	0.021 2	*
D^2	0.092 5	1	0.092 5	4.591 0	0.050 2	
Residual	0.282 1	14	0.020 1			
Lack of fit	0.242 7	10	0.024 2	2.466 2	0.199 3	
Pure error	0.039 3	4	0.009 8			
Sum of error	0.993 4	28				

$P > 0.05$ indicates that the model or factor is not significant; $0.01 < P < 0.05$ indicates that the model or factor is significant, represented by *, and $P < 0.01$ indicates that the model or factor was extremely significant, represented by **.

Effects of interaction of various factors on suspension stability of mango juice with skin Fig. 5 shows the response surface diagrams and contour diagrams of the interaction between sodium carboxymethyl cellulose, pectin, sodium alginate and xanthan gum on the suspension stability of mango juice with skin. As can be seen from the figure, the four response surfaces are hat-shaped with downward openings, and all of them have maximum values. The BD surfaces were steep and the rest slopes were gentle. The centers of the six contour maps are all located in the region of coded values, and the contour shapes are all close to ellipses, indicating that there was some interaction between factors, and factors B and D had a significant interaction. The centers of the four contour lines were all located in the areas of coded values, indicating that the optimal conditions for extraction were in the level ranges of the factors involved.

Result analysis It was concluded by solving and analyzing the extreme value of the model with software that the optimum formula of the compound stabilizer was sodium carboxymethyl cellulose 0.199%, xanthan gum 0.077%, sodium alginate 0.163% and pectin 0.118 5%, with which the theoretical stability was 74.60%. The stability obtained by the verification test was 70.32%, and the relative deviation (*RSD*) values from the theoretical value were less than 5%, indicating that the established stability regression model of mango juice with skin had high reliability.

Conclusions

In this study, mangoes were used as the raw material, and the effects of sodium carboxymethyl cellulose, pectin, sodium alginate and xanthan gum on the stability of mango juice with skin were discussed. According to the results of single-factor investigation, the optimum extraction process was determined by orthogonal experiment optimization and response surface methodology. The conclusions are given below.

① Through the orthogonal experiments of four stabilizers on the stability of mango juice with skin, the order of the main factors was sodium alginate (C) > sodium carboxymethyl cellulose (A) > pectin (D) > xanthan gum (B), and the optimum technical parameters were sodium carboxymethyl cellulose 0.20%, xanthan gum 0.08%, sodium alginate 0.18% and pectin 0.14%. With the above four optimal conditions, the suspension stability was 69.12%.

② In order to obtain the results from the response surface experiments of four stabilizers on the stability of mango juice with skin, the order of main factors was sodium alginate (C) > sodium carboxymethyl cellulose (A) > pectin (D) > xanthan gum (B), and the experimental results were consistent with the results of variance analysis. The optimum technological parameters were sodium carboxymethyl cellulose 0.199%, pectin 0.118 5%, sodium alginate 0.163% and xanthan gum 0.077%. With these conditions, the suspension stability was 70.32%.

③ Comparing the extraction rates obtained by the orthogonal method and response surface method, it was found that the stability of mango juice with skin obtained by response surface experiments was better than that obtained by orthogonal experiments.

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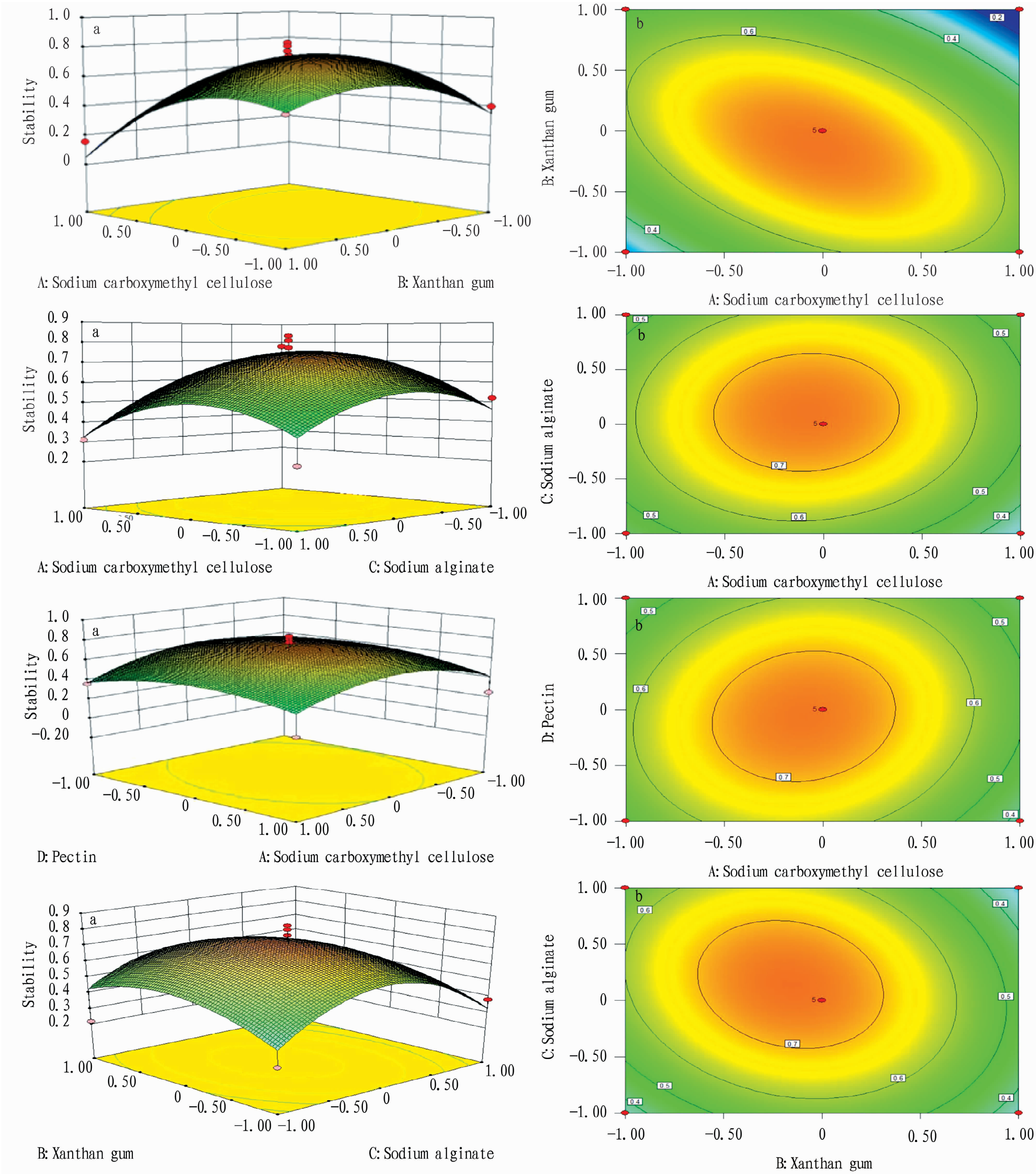

Fig. 5 Contour (a) and response surface (b) plots of the interaction of influencing factors on the stability of mango juice with skin

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU