Antioxidant Synergistic Effect and Formulation Optimization of Several Common Natural Pigments

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Abstract. Under DPPH system using the method of free radical scavenging, synergetic effect and optimal formulation of compound antioxidant among the solution of gardenia yellow, black bean red and sorghum red with the response surface method were determined. The results showed that, the component ratio of optimal compound antioxidant was gardenia yellow 20.00 μg ml^{-1} ,black bean red 100.00 μg ml^{-1},sorghum red 300.00 μg ml^{-1}, the DPPH scavenging rate of the system was 67.79%, compound antioxidant of gardenia yellow, black bean red and sorghum red had synergistic effect .Through the comparison of optimal mixed antioxidants and single antioxidant, the mixed antioxidants were better than single antioxidant slightly.

Introduction

Antioxidants, the free radical eliminators, are the important issue of research in the medical profession and antiaging field. If we absorb efficient antioxidants enough through food intake, it can help us remove the excess free radicals in our bodies, improve health, delay the disease and prolong life.

Gardenia yellow pigment is which is extracted from Gardenia jasminoides Ellis and widely used as a natural food colorant in Asian countries. Gardenia yellow are carotenoids of peculiar water-soluble behavior[1].Sorghum red pigment is from the sorghum shell and safety,not poisonous edible.Main components of red pigment were 5,4'–two hydroxyl isoflavones–7–O–galactoside and 5,4'–two hydroxyl–6,8–two methoxy isoflavone 7–O–galactoside[2].The black bean red belongs to the anthocyanins, which is the main component of delphinidin -3- glucoside and delphinidin and cyanidin -3- glucoside[5],Sorghum red pigment is isoflavones galactoside,a kind of natural pigment from the sorghum shell and safety,not poisonous edible. Main components of red pigment were 5,4'–two hydroxyl isoflavones–7–O–galactoside and 5,4'–two hydroxyl–6,8–two methoxy isoflavone 7–O–galactoside[3].The pigments natural pigments showed a higher scavenging activity against free radicals such as DPPH [1-3].

DPPH ·method is used to evaluate a natural antioxidant activity with sensitive, simple, rapid and feasible [4-5]. The gardenia yellow, black bean red and sorghum red as typical representative of natural pigments, the DPPH · scavenging rate as evaluation index of antioxidant activity, the synergetic effect and optimal formulation were determined using the response surface optimization method under DPPH ·scavenging system in this paper.

Material and Methods

Materials, Reagents

Black bean red pigment (content 27.29%) ,gardenia yellow pigment (content 13.60%) and sorghum red pigment (content 41.32%) were purchased from Qianjiang green sen treasure bio-tech Co., ltd.DPPH ·was purchased from Sigma company, other reagents were of analytic grade. Distilled

water was used throughout the experiment.

Antioxidant Test

DPPH radical-scavenging activity was performed by the method described of Ying QL [5]. The stock solution of DPPH ·was prepared by dissolving 25.63 mg in 100 ml absolute ethyl alcohol (6.5×10^{-4}mol/L). The stock solution was diluted to a dilution to 6.5×10^{-5} mol/L with 60% (v/v) ethanol for each determination. An aliquot of each dilution (1.0 mL) was mixed with solution of DPPH ·(5 mL, 6.5×10^{-5} mol/L). The mixtures were shaken vigorously and incubated at 37 ℃ in the dark for 30 min. A control containing 60% (v/v) ethanol (1.0 mL) and solution of DPPH (5 mL, 6.5×10^{-5} mol/L) was run at the same time. The absorbance was measured at 517 nm against 60% (v/v) ethanol (5.0 mL) mixed tested extract solution(1.0 mL)as a blank. The percentage of DPPH scavenging was calculated as follows: $P\%=[1-(Ai-Aj)/A_0] \times 100$, where Ai is absorption of tested extract solution, Aj is absorption of blank,A_0 is absorption of control.

Synergy Analysis Method

The effect produced exceeds the sum of their respective alone when used in combination of several substances, the synergistic effect is produced. The synergy evaluation method of antioxidant in vitro have three methods commonly: the response surface method, the addition method and the direct comparison method.RSM can optimize process parameters by combination of statistics and mathematics method and fit the factors and response value by quadratic regression equations[6].Comparison of the response coefficients in the equation, can be quantified analysis factors influence on the composite antioxidant activity[7].The subject chosen response surface methodology (RSM).

Response Surface Test Method

The Single Factor Test

The sample solution (1 mg/ml) was dissolved with water. The sample solution was mixed with DPPH solution (5 mL, 6.5×10^{-5} mol/L). To add a small amount the sample solution at first, increases gradually, and observe the fade of solution while still mixing. When the solution basic color faded, remembered the maximum concentration. Based on the maximum concentration, set up the appropriate gradient and make arithmetic sequence. According to the antioxidant test method of single factor tested.

The Response Surface Experimental Design

According to the single factor experiment results, response surface methodology experiments of Box-Behnken design were chosen for formulation optimization. The 3 factors and 3 levels were listed in Table 1.

Tab. 1 Levels of factors in response surface methodology

factors	levels		
	-1	0	1
A gardenia yellow [μg ml^{-1}]	20	30	40
B Sorghum red [μg ml^{-1}]	100	200	300
C Black bean red [μg ml^{-1}]	60	80	100

Result and Analysis

The Single Factor Test

The DPPH · Scavenging Rate of the Gardenia Yellow. Figure 1 showed, the ability of scavenging DPPH increased with increasing concentration in the 70 μg/ml range. The change of the

scavenging DPPH ability tended to be stable after 70 μg/ml. Three levels were of 20, 30, 40 μg/ml were determined at gardenia yellow concentration.

The DPPH ·Scavenging Rate of the Sorghum Red. Figure 1 showed, the ability of scavenging DPPH increased with increasing concentration and it had a high dose effect relationship in the range of 800 μg/ml. Three levels were of 100, 200, 300 μg/ml were determined at sorghum red concentration.

The DPPH ·Scavenging Rate of the Black Bean Red. Figure 1 showed, the ability of scavenging DPPH increased with increasing concentration in the range of 160 μg/ml. The change of the scavenging DPPH ability tended to be stable after 160 μg/ml. Three levels were of 60, 800, 100 μg/ml were determined at black bean red concentration.

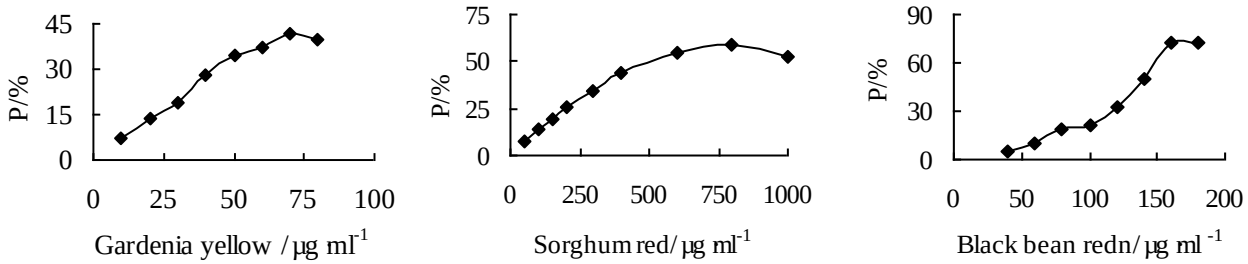

Fig.1 Effects of different factors on the DPPH ·scavenging rate

The Test Results of RSM

Based on the results of single factors experiments, the formulation optimization of gardenia yellow, black bean red and sorghum red experiments were carried out by RSM design according to Table 2.

Tab. 2 Data of RSM

No.	A	B	C	P/%
1	0	1	1	59.86
2	-1	0	-1	25.09
3	0	-1	1	51.18
4	-1	0	1	52.18
5	1	0	1	52.36
6	0	0	0	55.49
7	0	-1	-1	17.99
8	1	1	0	25.09
9	0	0	0	50.91
10	0	0	0	43.53
11	0	0	0	57.88
12	1	0	-1	42.15
13	0	1	-1	24.45
14	1	-1	0	56.91
15	0	0	0	53.3
16	-1	1	0	57.42
17	-1	-1	0	34.98

Analysis of Variance

Analysis of variance was carried out by design expert and the results showed that the p values of black bean red (C) ,the interaction among gardenia yellow(A) and sorghum red (B) were less than 0.01, which showed that the factors had most important influence.Analysis of variance showed that the factors and responses were not linear relationship. Above results showed that the specific factors and scavenging response did not have simple correlation relations.

Tab. 3 Analyze of mean square

Sourc of Squares	Sum of Square	Degree of freedom	Mean Square	F Value	Prob > F
Model	2680.64	9	297.85	5.03	0.0223
A	5.85	1	5.85	0.099	0.7624
B	4.15	1	4.15	0.07	0.7988
C	1401.85	1	1401.85	23.69	0.0018
AB	736.04	1	736.04	12.44	0.0096
AC	71.23	1	71.23	1.2	0.3089
BC	1.23	1	1.23	0.021	0.8893
A^2	17.24	1	17.24	0.29	0.6061
B^2	183.33	1	183.33	3.1	0.1218
C^2	221.53	1	221.53	3.74	0.0943
Residual	414.24	7	59.18		
Lack of Fit	293.11	3	97.7	3.23	0.1437
Pure Error	121.13	4	30.28		
Cor Total	3094.88	16			

Fitting the Model to Establish and Antioxidant Synergy Analysis

To fit the response surface test data, to obtain the coding variable regression equation of two order polynomia.

$$P=+52.22+0.85A+0.72B+13.24C-13.57AB-4.22AC+0.56BC-2.02A^2-6.60B^2-7.25\ C^2$$

The p vale of the lack of fit value was of 0.1437 (not significant), the model was of 0.0223 (significant), which showed that the model fit the RSM data significantly, the equation was fit the synergetic antioxidant effect among the gardenia yellow, black bean red and sorghum red.

According to the regression equation, the first degree were always positive, indicating that gardenia yellow,black bean red and sorghum red were positive correlation with the scavenging rate of DPPH : The interaction coefficient of gardenia yellow (A) and sorghum red (B) was negative value, reflecting the synergistic effect between two factors was negative cooperativity. Negative cooperativity effect of gardenia yellow (A) and black bean red (C) was in the same way.The interaction coefficient of sorghum red (B) and black bean red (C) were positive value, reflecting the synergistic effect between two factors was positive cooperativity. Compound antioxidant of gardenia yellow, black bean red and sorghum red had synergistic effect by and large .

Using the antioxidants concentration of gardenia yellow, black bean red and sorghum red as X and Y, the scavenging rate of DPPH as the Z, make the corresponding 3D figure. The interaction between gardenia yellow(A) and sorghum red (B) had great influence on the response, which showed relatively steep curve in the figures and it was consistent with the regression analysis results.

The Optimal Process Validation

The maximum value of model was calculated by the software. The equivalent linear regression model was shown in Figure 2.The component ratio of optimal compound antioxidant was gardenia yellow 20.00 μg ml^{-1}, black bean red 100.00 μg ml^{-1}, sorghum red 300.00 μg ml^{-1}, the DPPH ·scavenging rate was 67.79%, the verified value could reach above 67.5%, it was consistent with the predicted value and verified the validity of the model. The single component test with combination formulations of gardenia yellow 20.00 μg ml^{-1} , black bean red 100.00 μg ml^{-1},sorghum red 300.00 μg ml^{-1}, the actual production of DPPH ·scavenging rate were:13.62%%, 20.83% and 32.33%, respectively. The total scavenging rate was 66.78%,it was less than the combined formula slightly.It showed that combined formula antioxidant activity was better than single antioxidant and consistent with the regression model analysis.

Conclusion

In this study, in order to understand synergetic antioxidant effect of several common natural pigments among the gardenia yellow, black bean red and sorghum red, synergetic effect and optimal formulation of compound antioxidant were determined with the response surface method. The optimum formula of compound antioxidants were : gardenia yellow 20.00 g ml^{-1} ,black bean red 100.00 μg ml^{-1},sorghum red 300.00 μg ml^{-1}, the DPPH · scavenging rate was 67.79% under the conditions. Comparison the antioxidant activity between single antioxidant and compound antioxidants, concluded that compound antioxidants slightly better than single antioxidant. In the concentration, there were synergistic effect of antioxidant.

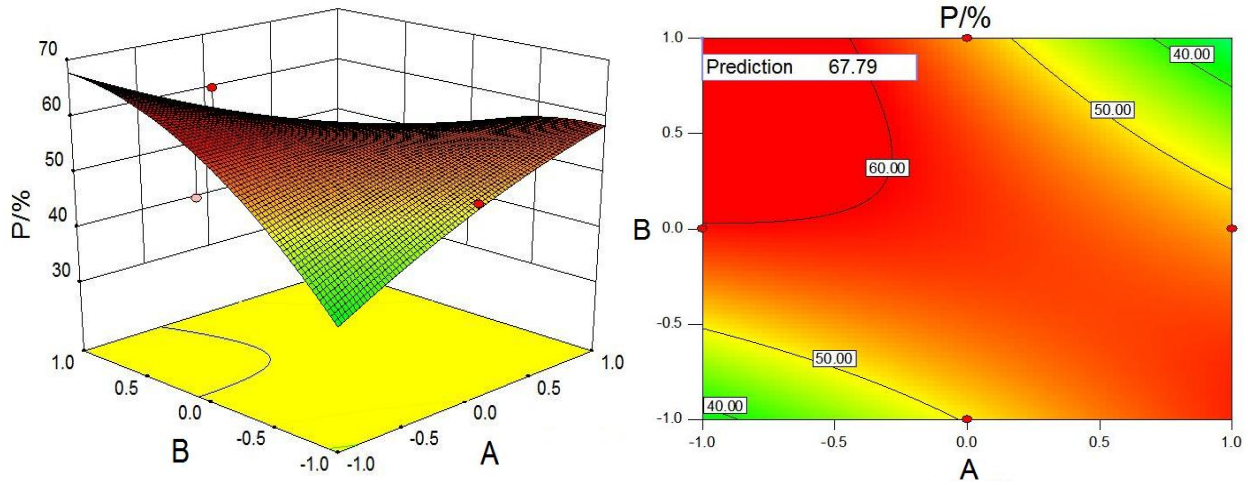

Fig.2 The equivalent linear regression model and P=f (A, B) response surface

Acknowledgments

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