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Research Article

Analysis of Influence Factors on Extraction Rate of Lutein from Marigold and Optimization of Saponification Conditions

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Abstract: After lutein esters extracted by ultrasonic-assisted organic solvent from marigold powder, saponification conditions such as saponification solution concentration, saponification lipuid dosage, saponification temperature and saponification time were optimized by response surface analysis. The results showed that the optimal saponification conditions are saponification solution concentration 10%, saponification lipuid dosage 200 mL, saponification temperature 50°C, saponification time 2 h. Under the optimal condition, the content of lutein is the highest with nalue of 15.64%.

Keywords: Extraction, lutein, marigold, saponification

INTRODUCTION

Marigold is commonly known as stink hibiscus, marigold, Tagetes Asteraceae and annual herb. Native to Mexico and it has been widely introduced throughout the world (Wang *et al*., 2004). In addition, as a general ornamental flower, it is not only rich in mineral elements, vitamins and other nutrients but also contains a variety of biologically active substances, to be fully utilized in various fields.

Lutein is a non-vitamin A active carotenoids (Mei, 1989), the system is named 3, 3-dihydroxy-a-carotene formula $C_{40}H_{56}O_2$, molecular weight 568.85 (Gou, 2002). As a natural pigment, with bright color, strong coloring, safe, non-toxic, strong antioxidant capacity characteristics, but also has to protect the eyes, cancer, modified UV damage, strengthen the immune system (Park *et al*., 1998), anti-atherosclerosis and other physiological functions. Widely used in feed additives, food additives, pharmaceutical, industrial dyes, aquatic products and other industries (Zhang *et al*., 2006). The study found mainly marigold lutein lutein esters (>90%) of the state of existence and lutein esters cannot be directly absorbed by the body metabolism, you must first be converted into free lutein can be absorbed by the body, so marigold extract lutein ester saponification, it is necessary to prepare a free lutein (Molldrem *et al*., 2004).

The author ultrasonic assisted extraction of petroleum ether on the basis of lutein esters, saponification with KOH methanol solution, based on the best conditions on the single factor experiment with SAS 9.1 software design response surface experiments, the results of response surface regression and analysis of variance of saponification conditions were optimized.

MATERIALS AND METHODS

Materials: Marigold particles are provided by Lindian Qi Xin natural products.

The main instruments are U-2001 UV spectrophotometer (Analytik Jena AG, Germany), RE-52A rotary evaporator (Haiya Wing Instrument), DHG-9053A-type electric oven thermostat blast (Yangzhou Hongdu Electronics Co. company), Nexus 670FT-IR fourier Transform infrared Spectrometer USA (Thermo Nicolet Corporation). The main reagents lutein standard sample (Sigma) and other reagents were of analytical grade.

Method:

Marigold lutein basic saponification process: After freeze-drying marigold pulverized particles passed through a 60 mesh sieve. Accurately weighed 30 g frozen brown powder into 500 mL volumetric flask, petroleum ether as the extraction agent, according to a certain ratio of solid to liquid extraction agent added, using ultrasonic assisted extraction method, filtered and the filtrate was concentrated under vacuum to obtain lutein ester solvent recovery Baptist paste; weighed accurately extract lutein esters 5 g, added mass of n-butanol dissolved in a certain amount of methanol was added a solution of KOH concentration saponification reaction was completed, filtration and the filtrate was measured lutein. The remaining filtrate was washed with HCI solution, pH adjusted 2.5 mol/L value to neutral and the filtrate was filtered with a separatory funnel,

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Table 1: Factors and their coded levels in central composite table

Level	X1KOH methanol concentration/%	X2 KOH amount of added methanol/mL	X3 saponification temperature/°C	X4 saponification time/h
1	7.5	100	45	1.5
0	10	150	50	2
-1	12.5	200	55	2.5

washed repeatedly with distilled water was added, the dark yellow lutein crystals are suspended in water, centrifuged, collected xanthophyll crystals, 50°C vacuum dried 24 h, to give lutein. The above operations are carried out under the exclusion of light.

Lutein determination method: Referring to AOAC (1970) Official Method 970.64-marigold particle content detection method.

Single factor experimental design: According to the basic flow, first determine the type of saponification liquid, then set each saponification concentration, dosage, saponification temperature and saponification time for the single factor experiments and they were repeated three times. Saponification concentration were 5, 7.5, 10, 12.5 and 15%, respectively a saponification liquid dosage is 100 mL, saponification temperature is 40°C, saponification time for the screening of optimum concentration of saponification conditions is for 2 h. At the optimum screening concentration of saponification, the amount of the saponification solution was 50, 100, 150, 200 and 250 mL, respectively saponification temperature was 40°C, saponification time was added for the screening of the optimal amount of the saponification conditions for 2 h. Under optimal screening concentration and amount of the saponification solution saponification temperatures were 35, 40, 45, 50 and 55°C, respectively saponification time optimal filter conditions for 2 h saponification temperature. Under the optimum screening concentration of saponification, the amount of temperature and saponification, saponification time optimal filter saponification time was 1, 1.5, 2, 2.5 and 3 h conditions, respectively.

Quadratic regression rotation combination experimental design: On the basis of single factor experiments, using SAS 9.1 software for response surface design of experiments, KOH methanol concentration selected, KOH methanol addition, saponification temperature, saponification time four factors multivariate cross combination experiments, respectively, X1, X2, X3 and X4 representatives, low, medium and high levels of each of the experimental variables, respectively -1, 0, 1 self-encoded. And the relationship between the true values encoded in line with the following equation:

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j$$

where, i is Encoded value of the argument, X_i is The actual value of the independent variable experimental level, X_0 is The level of the center of the actual value of the experiment, ΔX_i is The increase value of a single variable and Y is The lutein content of the response value, The experimental factor level coding is shown in Table 1.

Data processing and analysis: We use the SAS 9.2 software for quadratic regression rotation design process and analyze the experimental results.

RESULT ANALYSIS

Univariate results: Saponification solvent selection and concentration of the experimental results, the addition amount, the temperature of the saponification, the saponification time four factors obtained under different conditions lutein content results shown in Fig. 1.

Seen from Fig. 1A: A saturated solution of KOH in methanol was significantly better than the saturated ethanol solution of KOH in methanol and a saturated solution of NaOH, so the experiment was saponified select KOH solution in methanol solution.

Figure 1B shows: KOH methanol solution with increasing concentrations of lutein content after the first increase decrease. When the saponification solution concentration of 10%, the lutein content reaches the maximum value. Considering the various economic factors, etc., to determine the optimal concentration of 10% saponification.

Figure 1C shows: KOH methanol solution with increasing amount of lutein content increased. When the amount of KOH added 150 mL of methanol reaches a maximum, higher than 150 mL, lutein content of trans decreased and therefore to determine the optimal amount of methanol KOH 150 mL.

Figure 1D show: Saponification temperature increases, the lutein content increased. Saponification temperature is 50°C; the lutein content reaches the maximum and significantly higher than that of lutein other temperatures. When the saponification temperature is higher than 50°C, as the temperature rises, the content decreased. Therefore, to determine the optimum temperature of saponification 50°C.

Figure 1E shows: With the increase of the saponification time, lutein content increased. When the saponification time is 2 h we get the highest content of lutein. When the saponification time is greater than 2 h, lutein also declined. Therefore, we determine the best time for saponification is 2 h.

Results of quadratic regression rotation design: Quadratic regression rotation design experiments to

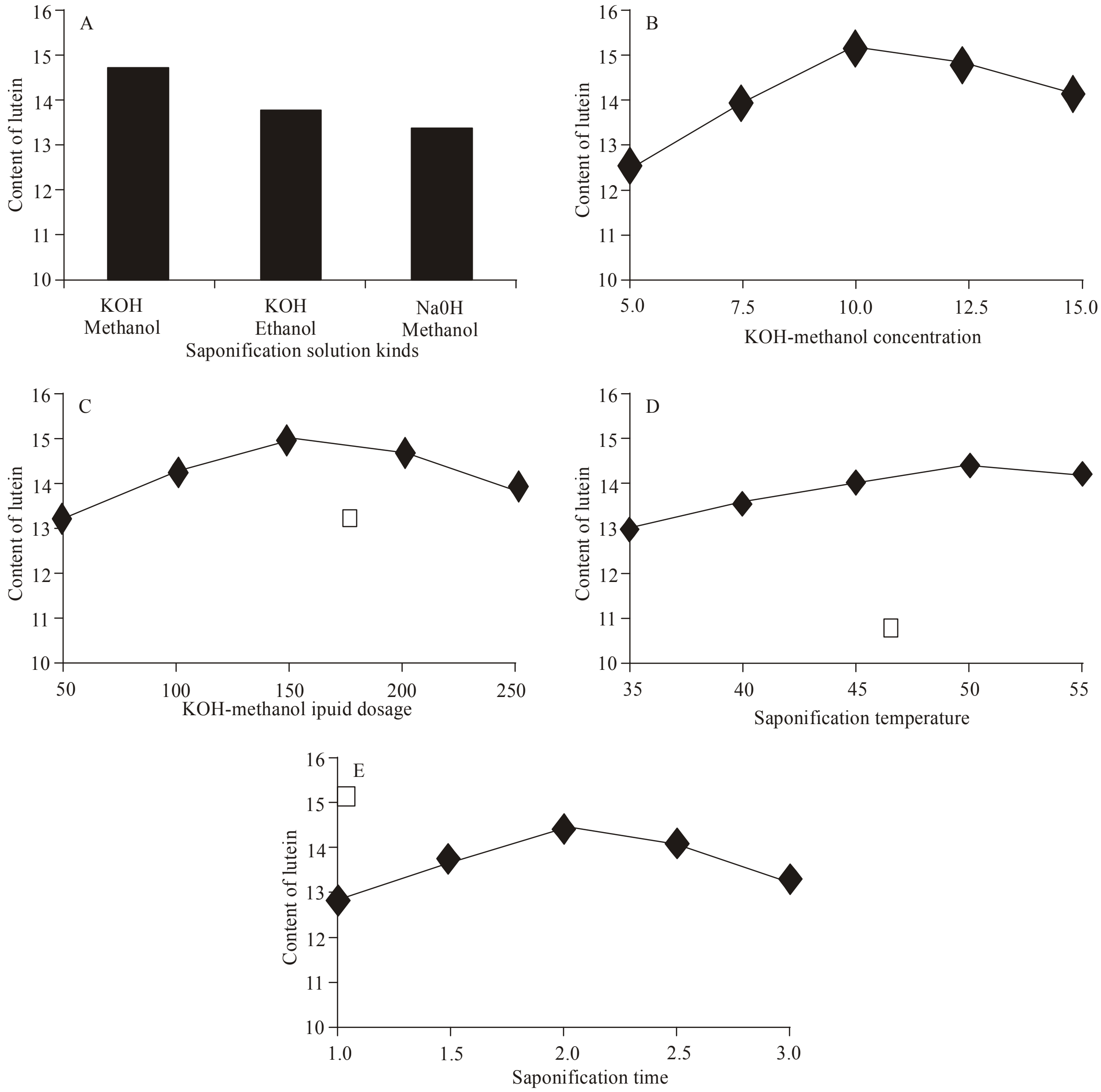

Fig. 1: Content of lutein under different saponification conditions

optimize the response surface method of saponification conditions are shown in Table 2.

Significance test of secondary regression equation: By F test to show the effectiveness of the regression equation, t test for regression model coefficients significant test, data processing system using SAS 9.2 on the experimental results of multiple regression analysis. The results are shown in Table 3.

Analysis of variance from Table 3 can be drawn: In a key, KOH concentration, KOH methanol addition, saponification temperature and saponification time were significantly affected ($p<0.05$) on the results. In the interaction term, the results between the four factors of significant influence. The quadratic regression model F is 16.52, greater than the F (14, 12) 0.01 = 4.05 and $p<0.0001$, while the lack of fit of F is 24.94, less than F (10, 2) 0.01 = 99.40, coefficient of determination $R^2 = 0.9507$, description and results of the model to be extremely significant. A term, the second term F values are greater than the level of F = 0.01, so this regression equation is valid, it can be predicted within the scope of the design used.

Table 4 shows the t-test analysis of variance: Factors affecting the interaction between X34 significant ($p<0.05$), X14 affect significantly ($p<0.01$). The degree of influence of various factors on the results in descending order of: X3, X1, X4, X2. The experimental data were SAS 9.2 statistical analysis, we can get four quadratic regression equation is as follows:

Table 2: Quadratic regression orthogonal rotation combination design and test results

KOH density X_1/%	KOH added methanol X_2/mL	Saponification temperature X_3/°C	Saponification time X_4/h	Lutein content Y/%
-1	-1	0	0	13.20
-1	1	0	0	13.55
1	-1	0	0	13.81
1	1	0	0	15.01
0	0	-1	-1	13.23
0	0	-1	1	14.71
0	0	1	-1	14.59
0	0	1	1	14.86
-1	0	0	-1	12.78
-1	0	0	1	14.75
1	0	0	-1	14.76
1	0	0	1	14.47
0	-1	-1	0	13.61
0	-1	1	0	14.80
0	1	-1	0	13.61
0	1	1	0	15.03
-1	0	-1	0	13.42
-1	0	1	0	14.72
1	0	-1	0	14.81
1	0	1	0	15.17
0	-1	0	-1	13.72
0	-1	0	1	14.41
0	1	0	-1	13.91
0	1	0	1	14.89
0	0	0	0	15.31
0	0	0	0	15.29
0	0	0	0	15.21

Table 3: Analysis of variance for the established regression model

Source	df	S.S.	M.S.	F	Pr>F
X1	1	2.622675	2.622675	44.707330	0.000100
X2	1	0.500208	0.500208	8.526783	0.012839
X3	1	2.784033	2.784033	47.457920	0.000100
X4	1	2.167500	2.167500	36.948210	0.000100
X1*X1	1	1.740408	1.740408	29.667810	0.000148
X1*X2	1	0.180625	0.180625	3.079017	0.104782
X1*X3	1	0.220900	0.220900	3.765564	0.076177
X1*X4	1	1.276900	1.276900	21.766630	0.000546
X2*X2	1	2.493408	2.493408	42.503790	0.000100
X2*X3	1	0.013225	0.013225	0.225439	0.643451
X2*X4	1	0.021025	0.021025	0.358402	0.560526
X3*X3	1	0.504300	0.504300	8.596532	0.012558
X3*X4	1	0.366025	0.366025	6.239432	0.028024
X4*X4	1	1.293633	1.293633	22.051870	0.000518
Model	14	13.570040	0.969289	16.522940	0.000100
LnRGDP	4	8.074417	2.018604	34.410060	0.000100
Quadratic term	4	3.416925	0.854231	14.561620	0.000148
Interaction	6	2.078700	0.346450	5.905747	0.004509
Error	12	0.703958	0.058663		
Lack of fit	10	0.698358	0.069836	24.941370	0.039147
Pure error	2	0.005600	0.002800		
Total error	26	14.274000			

S.S.: Sum of square; M.S.: Mean square

Table 4: t-test significance of each coefficient in the established regression model

Variable	Parameter estimate	S.E.	t	Pr> |t|
X1	0.467500	0.069919	6.686354	0.000100
X2	0.204167	0.069919	2.920066	0.012839
X3	0.481667	0.069919	6.888971	0.000100
X4	0.425000	0.069919	6.078504	0.000100
X12	-0.571250	0.104878	-5.446820	0.000148
X1X2	0.212500	0.121102	1.754713	0.104782
X1X3	-0.235000	0.121102	-1.940510	0.076177
X1X4	-0.565000	0.121102	-4.665470	0.000546
X22	-0.683750	0.104878	-6.519490	0.000100
X2X3	0.057500	0.121102	0.474805	0.643451
X2X4	0.072500	0.121102	0.598667	0.560526
X32	-0.307500	0.104878	-2.931980	0.012558
X3X4	-0.302500	0.121102	-2.497890	0.028024
X42	-0.492500	0.104878	-4.695940	0.000518

S.E.: Standard error

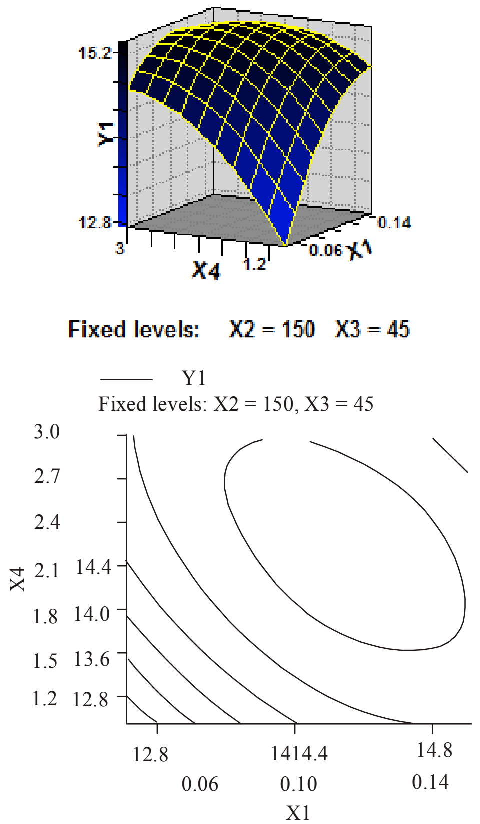

Fig. 2: Response surface and contour plot of concentration of KOH and the saponification time

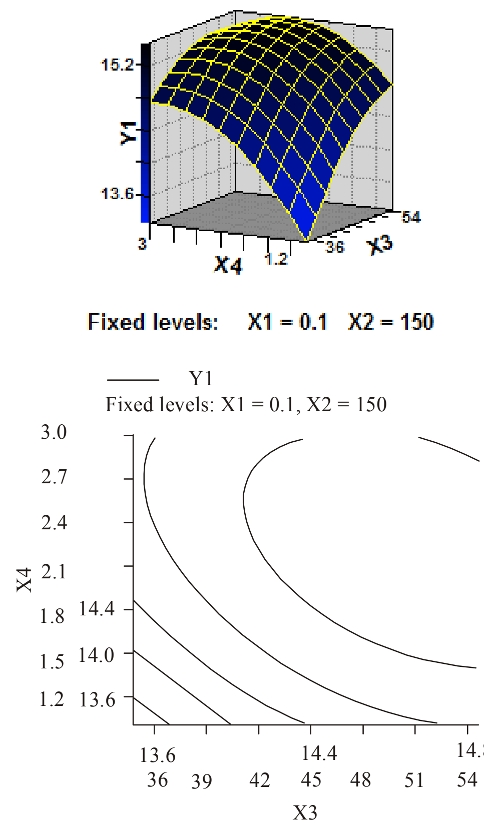

Fig. 3: Response surface and contour plot of KOH methanol addition and the saponification time

$$Y1 = 92.425 * X1 + 0.014267 * X2 + 0.423792 * X3 + 4.7775 * X4 - 228.5 * X1 * X1 + 0.0425 * X1 * X2 - 0.47 * X1 * X3 - 11.3 * X1 * X4 - 0.000068 * X2 * X2 + 0.000057 * X2 * X3 + 0.000725 * X2 * X4 - 0.003075 * X3 * X3 - 0.03025 * X3 * X4 - 0.4925 * X4 * X4 - 6.86344$$

Analysis of interaction: Dimension reduction assay using a fixed level of 0 in which two factors, the relationship between the two factors to obtain the results. Interaction equation is:

$$Y14 = -6.86344 + 92.425 * X1 + 4.7775 * X4 - 228.5 * X1 * X1 - 11.3 * X1 * X4 - 0.4925 * X4 * X4$$

$$Y34 = -6.86344 + 0.423792 * X3 + 4.7775 * X4 - 0.003075 * X3 * X3 - 0.03025 * X3 * X4 - 0.4925 * X4 * X4$$

According to the interaction equation, using SAS software can draw Y14, Y34 interaction response surface and contour equation.

Figure 2 shows: When KOH methanol addition 150 mL, when the saponification temperature 45°C, KOH concentration is increased to 10% from 6%, while the saponification time from 1.2 to 2.1 h also increased, along with the experimental results KOH concentration and increasing the saponification time increases. When the KOH concentration of 10%, the saponification time is 2.1 h, the maximum synergistic effect between the two, i.e., the result is maximized. When the KOH concentration from 10 to 14%, the extraction time from 2.1 to 3 h, the marigold lutein yield increases with the concentration and time of KOH saponification reduced, indicating that the two obvious antagonism.

Figure 3 shows: When the KOH concentration of 10%, KOH methanol addition is when 150 mL, saponification temperature increases from 36 to 50°C, extraction time increased from 1.2 to 2.1 h, the experimental results show with the saponification temperature increasing, the saponification time increases. When the saponification temperature is 50°C, saponification time is at 2.1 h, the synergy between the two maximum, optimal values of the experimental results. When the saponification temperature is 50 to 54°C, the saponification time from 2.1 to 3 h, the experimental results along with saponification time and increasing the temperature to reduce the saponification, indicating there existed significant antagonism.

Main effects analysis: The influence of various factors on the experimental results is shown in Table 5, various

Table 5: Analysis on the importance of key factor

V1	S.S.	df	M.S.	F
X_1	2.622675	1	2.622675	44.707330
X^2	0.500208	1	0.500208	8.526783
X_3	2.784033	1	2.784033	47.457920
X_4	2.167500	1	2.167500	36.948210

S.S.: Sum of square; M.S.: Mean square

Table 6: The results of some selected medium by flask-cultured experiments

Factor	Code	Real number	Max.	Real avg. number
KOH density	0.270826	0.113541	15.53	15.64
KOH adding	0.223793	172.379300		
Saponification temperature	0.655774	51.557740		
Saponification temperature	0.091205	2.091205		

Max.: Maximum; Avg.: Average

factors influence the sequence of: X3>X1>X4>X2, the impact on the experimental results of saponification temperature, followed by concentration of KOH and then is saponification time. KOH in methanol was added affect the amount of the minimum of the experimental results.

Optimal value selection and inspection: To find the optimal values of the regression equation, the results is shown in Table 6.

From Table 6, in response surface method predicted theoretical optimum extraction conditions do three validation experiments, the average content of lutein extract obtained was 15.53%.

In summary, the optimal combination of: KOH concentration of methanol is 10%, KOH methanol addition is 150 mL, saponification temperature is 50°C and saponification time is 2 h.

CONCLUSION

In this study, we took marigold particles as raw material, investigated the impacts of KOH methanol concentration, KOH methanol addition, saponification temperature and saponification time on the content of lutein. Through single factor experiments and four factors-three levels secondary rotation combination experiments to optimize the saponification conditions: KOH concentration of methanol is 10%, KOH methanol addition is 150 mL, saponification temperature is 50°C, saponification time is 2 h, lutein content of this condition is 15.64%. Verified experimentally derived results are consistent with the model predictions, indicating that this model can rely on the stability of the saponification conditions and good reproducibility.

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REFERENCES

AOAC, 1970. 64 Carotenes and Xanthophylls in Dried Plant Materials and Mixed Feeds Spectro-phometric Method. AOAC 45.1.04, AOAC Official Method 970, First Action.

Gou, J., 2002. Lutein and Its Application. J. Chongqing Norm. Univ., 19: 56-59.

Mei, Y., 1989. Encyclopaedia Sinica. Encyclopedia of China Publishing House, Beijing.

Molldrem, K.L., J. Li, P.W. Simon and S.A. Tanumihardjo, 2004. Lutein and beta-carotene from lutein-containing yellow carrots are bioavailable in humans. Am. J. Clin. Nutr., 80: 131-136.

Park, J.S., B.P. Chew and T.S. Wong, 1998. Dietary lutein absorption from marigold extract is rapid in BALB/c mice. J. Nutr., 128: 1802-1806.

Wang, G., H. Xu and S. Zhao, 2004. Progress in insecticidal plant marigold. J. Xinyang Norm. Univ., 4: 417.

Zhang, C., Q. Huang and G. Meng, 2006. Study abroad pigment marigold and deep processing products, production status. Inner Mongolia Agr. Sci. Technol., 38: 65-67.