

Optimization of parameters for extraction and conversion from color contain in achiote (*Bixa orellana* L.) into norbixin by response surface methodology

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Abstract:

Response surface method was used in this research to optimize the technical parameters of the extraction and transformation color compound in annatto seeds into norbixin. Parameters affected the extraction efficiency and norbixin recovery yield were solvent concentration, rate of solvent/seed, incubation time, and incubation temperature. The optimization results including: concentration of ethanol is 51.82⁰; concentration of NaOH is 0.52M; ratio of solvent/seed is 7.1/1; incubation time is 33.12 minutes, incubation temperature is 58.6⁰C, the rate of 36% HCl/color solution is 0.5/5 (v/v).

Keywords: Cellulose derivatives, Antibacterial activity, Shake flask method, MIC.

1. Introduction

Bixa orellana L. grows mostly in the tropics, is native to Central and South America, now widely grown in many tropical countries. Colonial period, *Bixa orellana* L. were imported to Vietnam, this plant grow wild or is cultivated in scattered in the South Delta, Central Highlands and central to harvest seeds, ornamental or medicine (Vo Van Chi, 1998). The color of Annatto compound that extracted by cashews'nut (*Bixa orellana* L.). Annatto is a natural color that has been widely used for a long time in the traditional cuisine of many nations in the world. In the food industry, Annatto natural color is the second most important behind the caramel and is recognized by CODEX as food coloring and color safe pharmaceuticals. Bixin and norbixin are two major compounds building to Annatto. Bixin is insoluble in vegetable oil. Norbixin soluble in water at high pH become yellow to orange colour. The chemical structure of bixin ester group should be able to convert bixin and norbixin in cashew colored sodium or potassium salt of norbixin to increase solubility in water (Le Thi Anh Dao et al, 2000; Nachtigall et al, 2009; Silva et

al, 2009), thereby increasing the usability of color annatto preparations.

2. Materials and Methods

2.1. Materials

Ripped annatto seeds were purchased in Vinh Cuu district, Dong Nai province. After shelling, removing the impurities, floaters and small grains, seeds were dried in an oven at temperature of 40⁰C until the moisture content reached about 10% as recommended by Mantovani group (2013).

- Acetone 99.7%; VN-Chemsol Co., Ltd.; Vietnam.
- Ammonia 25 ~ 28%; Guangdong Guanghua Chemical Factory; China.
- Chloroform 99%; Guangdong Guanghua Chemical Factory; China.
- CH₃COOH 99.5%; Guangdong Guanghua Sci-Tech Co, Ltd.; China.
- KOH 86%; Guangdong Guanghua Chemical Factory; China.
- 96% NaOH; Guangdong Guanghua Sci-Tech Co., Ltd.; China.
- Ethanol 99.5%; VN-Chemsol Co., Ltd.; Vietnam.

2.2. Research Methodology

2.2.1. Analysis of Norbixin

Sample was weighed as 0.25 g (± 0.02 g) (W) then dissolved by distilled water or appropriate solvent after that transferred into volumetric flask with a capacity of one liter. Next step, diluted by water or suitable solvent to the desired concentration, the concentration is depending on the concentration of the solution, and the last step, measuring the absorbance of the diluted solvent (A). (Distilled water or solvent were used as blanks)

$$\text{Pigment content (\%)} = 100 \times (A/A_{1\% \text{ 1cm}}) \times (F/W)$$

Among them:

- A: absorbance of the diluted solution
- $A_{1\% \text{ 1cm}}^{1\%}$: absorbance of standard solutions 1% (the thickness of cuvette is 1 cm)
- F: Factor of dilution (F = volume of diluted solvent / volume of standard solvent);
- W: volume of sample was diluted.

2.2.2. Evaluation method norbixin free recovery yield from extractive solvent

Add 5 g acid on extraction solvent, waiting deposited about 1 hour (Hyman et al, 1990) then centrifuging to collect the precipitate of norbixin after that washing the precipitate dissolved, dissolved by KOH solvent 0.5% to volume extraction initial translation (5 g). Dissolving with 0.5% KOH to measure the absorbance by wavelength at 482 nm (FAO JECFA, 2006). Extraction yield and norbixin recovery yield were calculated by the formula as below:

$$\text{Extraction yield (\%)} = (m_1 \times 100)/m_0$$

$$\text{Recovery yield (\%)} = (m_2 \times 100)/m$$

Among them:

- m: volume of Annatto contain in raw materials;
- m_0 : mass of dry matter contain in material;
- m_1 : mass norbixin be extracted;
- m_2 : mass of norbixin be extracted.

2.2.3. Assessment of solubility method

Following to Santos' method (Barbosa and coworker, 2005). Powder was stirred lightly in water as 0.4 revs per minute until become to a homogeneous solution then take note the time to dissolve. The time required less than 5 minutes was considered as good powder.

2.3. Conduct

2.3.1. Optimizing concentration of solvent by response surface method 2 factors (Central Composite Design - CCD).

After weigh 5g cashews, the sample was stirred in solvent about 30 revs per minute then incubating at 600°C for 30 minutes. After filling, take 1g solvent to 1000 mL of 0.5% KOH. Determine norbixin extraction yield (%) by measured at a wavelength of 482 nm. Experiments were illustrated by table number 1.

Table 1. Survey with 2 factors

Elements	Indicator variables				
	$-\alpha$	-1	0	+1	$+\alpha$
Ethanol (0)	21.72	30	50	70	78.28
NaOH (M)	0.36	0.4	0.5	0.6	0.64



Fig 1. Extracting and converting process into norbixin (a: stirring process, b: waste matter, c: extractive solvent was filtered, d: extractive solvent was incubated)

2.3.2. Optimization process ratio of solvent / material and conditions for saponification reaction by response surface method 3 elements (central composite design (CCD)).

The study was carried out as same as 2.3.1 item. The ratio of solvent/material, temperature ($^{\circ}\text{C}$), incubation time (minutes) were surveyed. Determine norbixin extraction yield (%) was measured by wavelength at 482 nm. Experiment processed by table number 2.

Table 2. Survey with 3 factors

Elements	Survey				
	$-\alpha$	-1	0	+1	$+\alpha$
Ratio of solvent /material	2.64	4	6	8	9.36
Incubation temperature (^0C)	43,2	50	60	70	76.8
Incubation time (min)	13.18	20	30	40	46.82

2.2.3. Survey volume of concentrated chlorhydric acid to precipitate free norbixin

Weigh 5 g of the solution was extracted then drip Solid Acid 36% in (Solid Acid was dripped slowly into a centrifuge tube (Haque, 2011) volume of centrifuge are 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6 and 0.65 mL, waiting for 1 hour and then centrifuged precipitate collected. Wash precipitate several times with distilled water. Dissolve the precipitate by KOH 0.5% into 5 g. Taking 1 g dilute to 1000 mL of 0.5% KOH. Determine the norbixin free recovery yield (%) by wavelength at 482 nm.

2.2.4. Design and Data Processing

The experimental were designed by software JMP 9.0.2 program (SAS Institute Inc., 2011, USA), analysis of variance ANOVA, comparing average by Tukey's HSD method.

3. Results and Discussion

3.1. Optimization concentration of solvent

Result of extraction efficiency and color conversion in center point are higher than others. This is a sign that the optimum point can be located near the center survey.

Table 3. The result of experience

Variable	Encryption	X ₁ – concentration of ethanol (°)	X ₂ – concentration of NaOH (M)	Extraction of norbixin yield (%)
1	---	30	0,4	1,14
2	-+	30	0,6	1,28
3	+ -	70	0,4	1,3
4	++	70	0,6	1,42
5	a0	21,72	0,5	0,98
6	A0	78,28	0,5	1,11
7	0a	50	0,36	1,18
8	0A	50	0,64	1,46
9	00	50	0,5	1,78
10	00	50	0,5	1,74
11	00	50	0,5	1,72
12	00	50	0,5	1,7
13	00	50	0,5	1,8

Influence level of these factors and interactions each other on the response Y is illustrated and arranged on a Pareto chart (Figure 2). Accordingly, influence level extraction efficiency yield and color conversion into norbixin descending as X12 interactions have the greatest impact,

followed by interactive X22, the third is the concentration of NaOH (X2), after the concentration of ethanol(X1) and finally the interaction X1X2 not significantly affected. Thus, if you compare between ethanol and NAOH, the effect of ethanol is important than NaOH

Term

- X1- concentration of ethanol * X1- concentration of ethanol
- X2- concentration of NAOH (M) * X2- concentration of NAOH (M)
- X2- concentration of NAOH (M) (0.4 ; 0.6)
- X1- concentration of ethanol (M) (30 ; 70)
- X1- concentration of ethanol * X1- concentration of NAOH

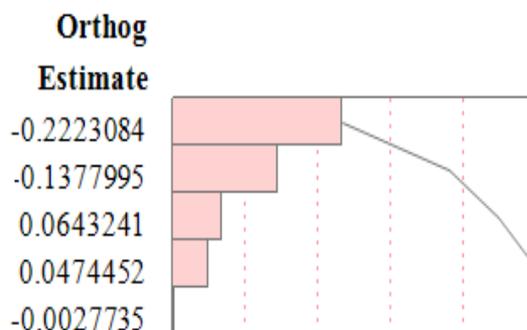


Fig 2. Pareto chart showing the effects of these factors

The relationship of the response Y - extraction yield X1 - ethanol concentration X2 - concentration of NaOH was demonstrated by a equation as: $Y = 1.748 + 0,06X1 X2 + 0.082 - 0.326 X12 - X22 .188$. Response surface showing the influence of the concentration of two-component solvent extraction performance and norbixin convert color to look like in Figure 3. Surface above the peak point, is extraction efficiency and norbixin color transformation into the highest ranges survey.

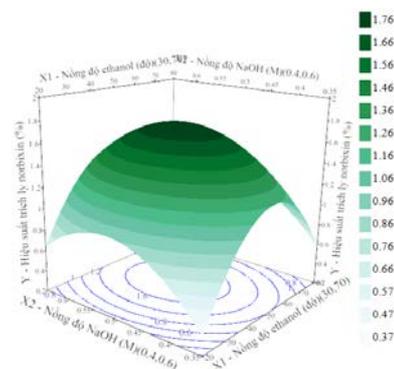


Fig 3. Response surface showing the relationship between yield and concentration of two ingredients solvent

Optimal results are obtained optimal optimum concentration of ethanol (51,820) and NaOH (0.52 M) will be used to conduct subsequent experiments as a basis for the construction and manufacturing processes.

conditions saponification

After conducting experiments with random order, the results are summarized in Table 4.

3.2. Optimized ratio of solvent / material and reaction

Table 4. Experimental results 3 CCD elements

Variable	Encryption	X ₁ – Ratio of solvent / material	X ₂ – Incubation temperature (°C)	X ₃ – Incubation time (min)	Y – Extraction of norbixin yield (%)
1	---	4	50	20	1,03
2	--+	4	50	40	1,17
3	-+-	4	70	20	0,83
4	-++	4	70	40	0,98
5	+--	8	50	20	1,46
6	++-	8	50	40	1,57
7	++-	8	70	20	1,34
8	+++	8	70	40	1,46
9	a00	2,64	60	30	0,85
10	A00	9,36	60	30	1,59
11	0a0	6	43,2	30	1,38
12	0A0	6	76,8	30	1,28
13	00a	6	60	13,18	0,98
14	00A	6	60	46,82	1,6
15	000	6	60	30	1,8
16	000	6	60	30	1,69
17	000	6	60	30	1,78
18	000	6	60	30	1,82
19	000	6	60	30	1,75
20	000	6	60	30	1,82

Overall, the results in Table number 4 show that extraction efficiency and color conversion into the norbixin (from 15 to 20 treatments) at the solution in central is the best. This is a sign that the optimum point can be located near the center survey. Influence level of these factors and their interactions on the response Y is illustrated and arranged on a Pareto chart (Figure 4). Accordingly, extraction efficiency and color conversion into norbixin as follows: X1 - the ratio of solvent / Most affected grain, X32 interaction affects less than second place,

followed by interactive X12 and X22, then the annealing time and temperature of incubation X2 X3, 3 pairs of interacting pairs of 3 factors significantly influence the performance extracted and converted into norbixin color. Thus, if you compare the effects of three factors, the impact of the rate of extraction efficiency and color conversion into the more important norbixin incubation time, incubation temperature was at number 3 most influential factors in survey

Term

- X1 - Ratio of solvent/material (4/8)
- X3 - Incubation time (min)* X3 - Incubation time (min)
- X1 - Ratio of solvent/material * Ratio of solvent/material
- X2 - Incubation temperature (°C)* X3 - Incubation temperature (°C)
- X3 - Incubation time (min) (20.40)
- X2 - Incubation temperature (50.70°C)
- X1 - Ratio of solvent/material * X2- Incubation temperature
- X1 - Ratio of solvent/material * X2- Incubation time (min)
- X2 - Incubation temperature (50.70°C)* Incubation time (min)

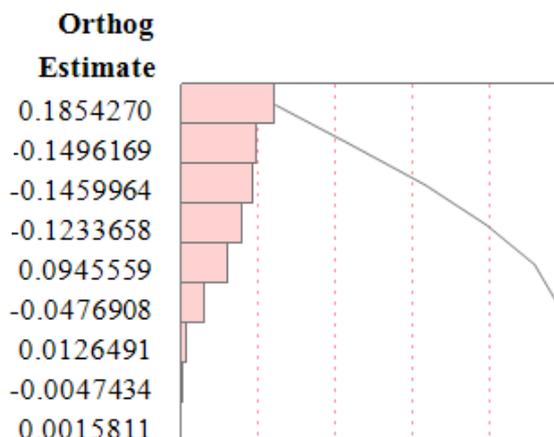


Fig 4. Pareto chart showing the effects of these factors

The results obtained can represent the relationship between the response Y - Extraction

yield and convert color into 3 elements norbixin with X1 - ratio solvent / particle, X2 - X3 and annealing temperature - incubation time by the following regression equation: $Y = 1.777 + 0.224 X1 - 0.058 X2 + X3 0.114 - 0.201 0.162 X12 - X22 - X32 0.176$. Response surface corresponding to each

pair of elements is shown in Figure 5, the response surface on the top side, ie the highest performance ranges.

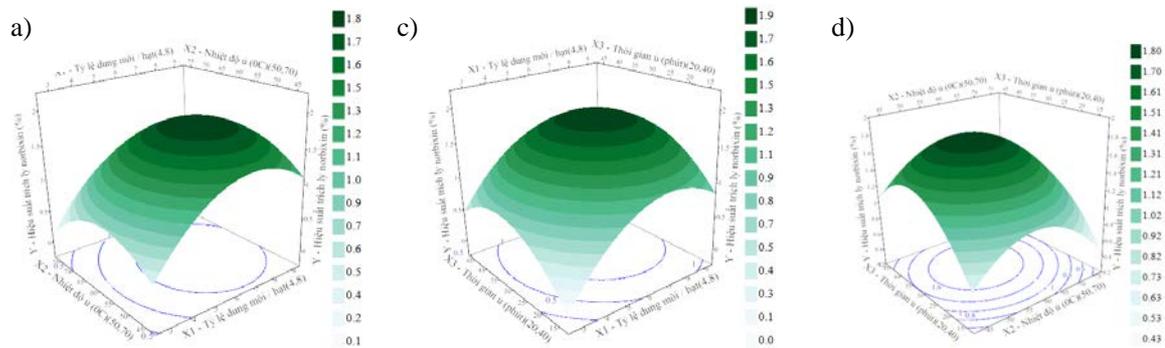


Fig 5. Response surface showing the correlation between the extraction efficiency between: a) annealing temperature and the ratio of solvent / seed; b) incubation time and the ratio of solvent / seed; c) annealing temperature and time

The result is optimum solvent ratio / seed is 7.1 / 1, 58.58 °C annealing temperature and annealing time is 33.12 minutes, while the extraction efficiency and color conversion into norbixin estimated 1,86%; equivalent to 86.92% recovery efficiency. Optimal parameters are consistent with the recommendations of Nachtigall et al. (2009) that the implementation of the saponification reaction of bixin norbixin should not raise the temperature too high for a long time, the temperature should be below 70 0C and shorter time to 1 hour.

3.3. Effect of hydrochloric acid concentration to precipitate free norbixin

The survey of the impact of volume of 36% HCl acid on norbixin retrieval performance is shown in Figure 6

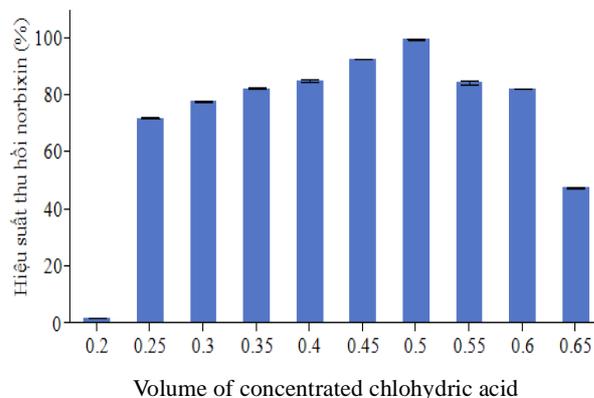


Fig 6. Influence of volume of 36% chlohydric acid recovery performance norbixin

From the average results compare with Tukey's HSD method showed that the volume of concentrated hydrochloric acid precipitates the best

norbixin 0.5 mL and mean differences with retrieval performance when using different volumes of HCl in p = 0.05 level. With the amount of fluid extracted in each treatment is 5 g (5.43 mL), then 0.5 mL of 36% HCl when dissolved in translation extract equivalent to a concentration of 1 M. This result is consistent with the method of Dinda et al. (2008) when the authors use dilute hydrochloric acid to precipitate norbixin obtained after solvent extraction with 5% KOH.

3.4. Solubility of the powder precipitated at pH norbixin in different countries

Using 0.1 g norbixin precipitated dough for each treatment to the survey conducted in water solubility of precipitated dough norbixin at different pH value, the results shown in Figure 7.

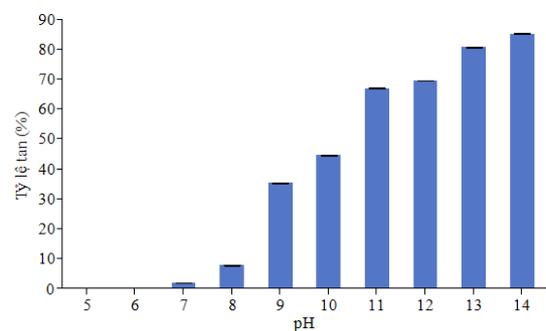


Fig 7. Norbixin ratio dissolved in water at different pH

After comparing the average rate of melting of norbixin at different pH by means of Tukey's HSD showed that only at pH 5 and 6 percentage tan difference not significant, the remaining percentage tan differences significant pairs. Thus norbixin precipitated powder soluble in water at a pH below 7, while at pH above 7, the melting rate proportional

to the pH value. Experimental results with the correct reference information about the solubility of the precipitate norbixin powder in water (Dinda et al., 2008), the reason is freedom in powder precipitated norbixin have long hydrophobic carbon vessels, namely HLB value is 2.65 lower than 7

4. Conclusion

Annatto Colour in Vietnam's cashew color can be extracted and converted into ethanol solvent norbixin alkaline by NaOH. The recovery of 87% at best in the conditions as follows: ethanol concentration of 51,820; concentration of 0.52 M NaOH; ratio of solvent / material is 7.1 / 1; annealing temperature and time respectively 58.6 0C and 33.12 minutes. After extraction, norbixin freedom can be separated from the service by means of acid extraction chemistry. Concentration of 1 M hydrochloric acid (HCl or 9.2% volume concentration versus fluid volume extracted) is best suited to precipitate withdrawal norbixin freedom. After drying at 50 0C precipitate obtained powder precipitated with 44.25% norbixin.

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