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Different factors influencing to annatto dye extraction in *Bixa orelana* L. seeds

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Abstract
Annato is redish orange in colour, usualy soft, but hard and britle when dry. It has a peculiar swetish odour and a disagreable saline biterish taste. It softens in water, to which it imparts a yellow color, but does not disolve. The principal pigment in annato extract is bixin, which is contained in the resinous coating of the seed itself. Annato sems to be an important natural colorant for fod and drug industries owing to its potential uses as a substiute for Tartrazine which is a synthetic colourant that is prohibited in many countries. Purpose of our research is to investigate some major factors affecting to annatto dye extraction from *Bixa orelana* L. seeds such as kind and concentration of extraction solvent; ratio of solid/solvent; extraction time and temperature. Our results are as follows: solvent NaOH 0.5 M, ratio of solid/solvent 5g/90ml, extraction time 5 hours at temperature 80 ^{o}C.

Keywords: Bixa orelana, dye extraction, solvent, annatto.

1. Introduction
Bixa orelana L. is an ancestral multiuse plant popularly known as Achiote or lipstick tree in view of its redish – orange dye on its seeds. The dye obtained from a thin, highly coloured resinous coating of triangular seeds present in brown or crimson capsular fruit is caled as "annato" colourant (P. Giridhar *et al.*, 2014) [8]. Its seeds are composeed of an 'iner seed' with a sheled kernel containing oils, waxy substances, mineral ash and alkaloid compounds, a pel compriseed of celulose and tanins, and an outer cover containing pigments, moisture, and a smal amount of oils (Ribeiro JA, *et al.*, 2005) [17]. Bixin, an apocarotenoid devoid of pro-vitamin A activity, is the main oil soluble pigment found in annato (McKeown GG *et al.*, 1962) [11]. Hydrolysis of the bixin methyl ester group yields the dicarboxylic acid, norbixin, which is an annato pigment soluble in aqueous alkaline solutions (McKeown GG *et al.*, 1962; Reith JF. Gielen JW, 1971) [11, 16].
Annato has ben aplied to the production of various fods. In particular, the oil-soluble annato colour is useed in dairy and fat-baseed products like buter, margarine, chese, baked and snack fods, and also in pharmacy, dyeing of leather and cosmetics (Scotter MJ, 1998) [20]. Annato color imparts yelow to red with varied hue index as it poseses high tinctorial value, hence have signifcance in the fod industry as a natural fod grade colour, and stands second in rank among economicaly important natural fod colourants, apart from its wide use in some regions of the world for non-fod aplications viz., to color textiles (Lata R *et al.*, 1990) [10], fabrics and weapons (Rao PGP et al;, 2002) [15]. Several articles on *Bixa* provided a brief information about annato chemistry (Preston HD *et al.*, 1980) [14], its extraction methods and formulations (Aparnathi KD *et al.*, 1991) [3], pharmacology (Srivastava A *et al.*, 1999) [22], its toxicology and procesing (Satyanarayana A *et al.*, 2003) [18] and methods to analyze its colour (Scotter MJ. *et al.*, 2009) [21]. The quality of seeds and their geographical conditon to had influence on annato dye yield as evident from various reports wherein, the seeds are the best with 3-4% bixin content. (Chuyen *et al.* 2012) [6] have demonstrated improvement in bixin extraction yield, and also extraction quality from annato seed by modifcation and combination of diferent extraction methods. In another study (Ribeiro JA *et al.*, 2005; Albuquerque CLC, Meireles MAA, 2012) [17, 1], researchers have aplied supercritcal CO_2 method as a pretreatment for defating of annato seeds. reduce the serum cholesterol concentration by reducing the intestinal absorption of dietary. Irespective of the method of extraction either using oils or using solvents, bixin can be hydrolyzed into norbixin under specifc conditons of temperature and pH, the dicarboxylic acid and saponifed into the potasium salt of norbixin. At elevated temperature (>70 OC), annato pigment gets degraded

and form several products including a 17-C yellow compound known as McKeown's pigment [28]. Supercritcal extraction with CO2 could be a god alternative to avoid these problems (Mendes RL *et al.*, 2003) [12]. Studies of Annato pigment extraction have ben caried out using supercritcal CO2 (Degnan AJ, *et al.*, 1991; Chao RR *et al.*, 1991; Silva GF *et al.*, 2008) [7, 5, 19] and CO_2 modifed with several entrainers (methanol, chloroform and acetonitrile) (Anderson SG *et al.*, 1997) [2]. It was shown that the entrainers increaseed the eficiency of extraction.

The stabilty of the aded annato dye in fods is the most important parameter which is esential especialy from quality and aesthetic point of view. Though bixin part of annato pigment is highly stable compared to other carotenoids such as betacarotene, etc., which is mainly due to its apocarotenoid nature, various studies revealed that bixin to is susceptible to procesing and storage conditons especialy to high temperatures and light which leads to a loss in the color of the annato aded foods (Bersetj C *et al.*, 1986; Najar SV *et al.*, 1998) [4, 13]. Similarly the efect of water activity is reported to be having influence on bixin stabilty, wherein, bixin is more stable at intermediate and higher water activites (Gloria MBA *et al.*, 1995) [9].

Purpose of our research is to investigate different factors affecting to annatto dye extraction from *Bixa orelana* L. seeds such as kind and concentration of extraction solvent; ratio of solid/solvent; extraction time and temperature.

2. Material & Method

2.1 Material

Bixa seed is originated from Mekong river delta, Vietnam. The seed is collected from wild-growing bushes or from plantations.

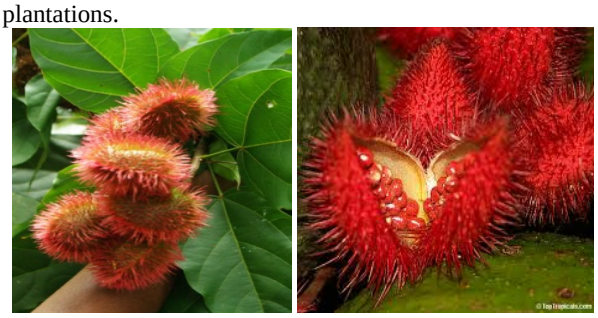

Fig 1: *Bixa* seed

2.2 Research method

2.2.1 Examine kind and concentration of solvent for extraction

Boiling 10 samples (each sample 5 gram) with 100 ml NaOH and KOH with following concentrations: 0.05 M; 0.1 M; 0.5 M; 1.0 M; 1.5 M at temperature 90 ^{0}C. After that we filter the extracted fluid, take 0.5 ml filtrate to measure by UV – VIS. Acidify the remained filtrate with 100 ml HCl 3M, filter to get the particle. Take filtrate to drying in glass oven in 50oC. Compare the dye with the initial weight.

2.2.2 Examine the ratio of solid/solvent

Boiling 5 samples (each sample 5 gram) with NaOH 0.5M: 60ml, 70ml, 80ml, 90ml, 100ml at temperature 90^{o}C in 4 hours. After that we filter the extracted fluid, take 0.5 ml filtrate to measure by UV – VIS. Acidify the remained filtrate with 100 ml HCl 3M, filter to get the particle. Take filtrate to drying in glass oven in 50 ^{0}C. Compare the dye with the initial weight.

2.2.3 Examine extraction time

Boiling 5 samples (each sample 5 gram) with 90 ml NaOH 0.5 M at temperature 90^{o}C in different duration: 3h, 4h, 5h, 6h, 7h, 8h, 9h. After that we filter the extracted fluid, take 0.5 ml filtrate to measure by UV – VIS. Acidify the remained filtrate with 100 ml HCl 3M, filter to get the particle. Take filtrate to drying in glass oven in 50^{o}C. Compare the dye with the initial weight

2.2.4 Examine extraction temperature

Boiling 5 samples (each sample 5 gram) with 90 ml NaOH 0.5 M in 5 hours at different temperatures: 60 ^{0}C, 70 ^{0}C, 80 ^{0}C, 90 ^{0}C, 100 ^{0}C. After that we filter the extracted fluid, take 0.5 ml filtrate to measure by UV – VIS. Acidify the remained filtrate with 100 ml HCl 3M, filter to get the particle. Take filtrate to drying in glass oven in 50 ^{0}C. Compare the dye with the initial weight

2.3 Statistical analysis

All data are processeed by Excell 2003.

3. Result & Discussion

3.1 Effect of kind and concentration of solvent to annatto extraction

Table 1: Optical density of annatto extracted by different solvent concentrations

λ (nm)	Optical density (A)									
	NaOH (M)					KOH (M)				
	0.05	0.1	0.5	1.0	1.5	0.05	0.1	0.5	1.0	1.5
453	1.2509	1.4731	1.5701	1.4281	1.0968	0.8809	1.4112	1.5313	1.3240	1.2221
481	1.0395	1.1989	1.3051	1.1673	0.8468	0.7257	1.1557	1.2571	1.1214	0.9691

Table 2. % Dye extracted by different solvents

Solvent	NaOH (M)					KOH (M)				
	0.05	0.1	0.5	1.0	1.5	0.05	0.1	0.5	1.0	1.5
M_0(g)	5.004	5.006	5.012	5.012	5.007	5.007	5.009	5.009	5.011	5.008
M_1(g)	1.958	1.963	1.871	1.889	1.915	1.950	1.852	1.865	1.903	1.862
M_2(g)	2.339	2.462	2.455	2.380	2.255	2.237	2.308	2.412	2.304	2.221
%Dye	7.614	9.968	11.652	9.798	6.790	5.732	9.104	10.920	8.002	7.169

From table 1 and table 2, we see that NaOH 0.5M and KOH 0.5M extract annatto higher than other concentrations. Meanwhile, extraction by NaOH 0.5M shows the optical density at two wavelength 453nm, 481nm; the dye extracted is higher than sample extracted by KOH 0.5M. Moreover, ion Na^+ is healthier than ion K^+, NaOH is more economic. So we choose NaOH 0.5M to extract annatto from *Bixa orelana* L.

3.2 Effect of ratio solid/solvent

Table 3: %dye extracted by different solvent volumes

V_{NaOH} (ml)	60 ml	70 ml	80 ml	90 ml	100 ml
M0 (g)	5.012	5.010	5.013	5.010	5.008
M1 (g)	1.869	1.903	1.869	1.819	1.863
M2 (g)	2.396	2.466	2.467	2.460	2.504
%dye	10.515	11.238	11.929	12.794	12.799

From table, when we use more NaOH 05 M, the dye extracted is also increased respectively, especially from 60 ml to 90 ml. So we choose solvent volume 90 ml for further experiments.

3.3 Effect of extraction time

Table 4: Optical density of extracted fluid by different extraction times

λ (nm)	Optical density (A)						
	3h	4h	5h	6h	7h	8h	9h
453	1.1280	1.3035	1.4094	1.1453	1.1158	1.0402	0.8654

We acidify the extracted fluid by 100 ml HCl 3M, dry the filtrate at 50 ^oC, calculate the % dye.

Table 5: % dye by different extraction times

Time (h)	3h	4h	5h	6h	7h	8h	9h
M0 (g)	5.007	5.006	5.001	5.006	5.004	5.003	5.000
M1 (g)	1.851	1.810	1.935	1.790	1.822	1.765	1.823
M2 (g)	2.455	2.452	2.615	2.405	2.389	2.304	2.271
% dye	12.063	12.825	13.597	12.285	12.063	10.774	8.960

From table 4 and table 5, when we increase the extraction time we shall get more percentage of dye. However if we increase the extraction time (from 6h to 9h) the extraction recovery shall be down because the high temperature and long time can cause damage to annato. So we choose the extraction time 5h for further experiments.

3.4 Effect of extraction temperature

Table 6: Optical density of extracted fluid by different extraction temperatures

λ (nm)	Optical density (A)				
	60°C	70°C	80°C	90°C	100°C
453	1.2880	1.4305	1.4523	1.3766	1.2642

Table 7. % dye of extracted fluid by different extraction temperatures

Temperature	60°C	70°C	80°C	90°C	100°C
M0 (g)	5.008	5.002	5.010	5.011	5.009
M1 (g)	1.875	1.803	1.889	1.792	1.859
M2 (g)	2.453	2.455	2.619	2.477	2.496
% dye	11.542	13.035	14.571	13.670	12.717

From table 6 and table 7, we see the high percentage of annato dye if the sample is treated at temperature 60 ^0C-80 ^0C. If we continue increasing the extraction temperature to 90 ^0C, 100 ^0C; the percentage of dye extracted shall be decreased owing to thermal damage. So we choose the optimal temperature 80 ^0C for annato extraction.

4. Conclusion

Annato is obtained from the thin resinous aril portion of seeds of Bixa orelana - a tropical plant of great agroindustrial interest. Bixin and norbixin are the main components of annatto colour which imparts red to yelow hue to the fod matrix. Annato is the most sought after natural colorant in the fod industry in view of its availabilty, afordabilty and viabilty. It also finds wide use in cosmetics, pharmacy and dyeing purposes. We have successfully determined some main technical factors influencing to the annatto extraction such as kind and concentration of solvent, ratio of solid/ solvent, extraction time, extraction temperature.

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