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Economically Sustainable Production Methodologies for Food Grade Colors from *Bixa orellana* Seed

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ABSTRACT

Food colorants isolated from seeds of *Bixa orellana* or annatto appears as an unique one for food and drug industries owing to its potential uses. It has a very high commercial and medicinal value in domestic and international markets. This annatto dye is orange red and deep red colored dye present as a thin layer with wax, gum and some other very few compounds on the seed coat of mature fruit. This is nontoxic natural food color insoluble in water but soluble in edible oil and some organic solvents. The principal color pigment in annatto dye is an appocaretenoid molecule known as bixin. Though bixin is insoluble in water but can be converted into water soluble form by careful hydrolysis. These water-soluble forms can be isolated from other associated material by several physical means. As food additives particularly we need both water and edible oil soluble forms. Naturally annatto dye is water insoluble but insoluble in edible oils. So, our objectives of present research work were to separate bixin from other impurities, make it water soluble and considerably stable form for practical use. Accordingly, economic and health friendly extraction and isolation procedures for bixin from seed coat were developed. Economic and health friendly procedures for conversion of bixin into water soluble form and also solid from were developed. Isolated bixin solid is edible oil soluble form. The toxicity of the product was investigated in authorized institute. No toxicity was found. Presence of toxic metallic content in the product was also investigated. All the metals were found to below the international permissible limits for human being. So economically sustainable and health friendly technology for different forms of annatto food colors has developed in the present research work. Thin layer chromatographic study, Ultra violet (UV) and Infrared spectroscopic of the products were also carried out to identify the compounds and their functional nature.

Introduction

Over the past few decades, it has been a growing concern worldwide for food quality, safety and security. Particular attention has been drawn to food colorants used in food, drug, cosmetics and textile industries, where the safety of colorants is one of the most burning issues. Synthetic food colors are seriously injurious to health and now a days are serious concern for human health but natural food colors are not harmful to health and also beneficial to it. *Bixa Orellana* is a typical medium sized plant and is well suited for planting in the country like Bangladesh, India, Srilanka, Central and South America, Java etc. This plant is valued for edible orange red and deep red colored dye present as a thin layer with some wax, gum and few other compounds on the seed coat of mature fruit. This is a non-toxic natural food color insoluble in water but soluble in edible oil and in some organic solvents. The common international name of this dye is annatto. Annatto is known for its lack of toxicity, its high tonsorial value and its high range of color-comprising of red, orange and yellow hues (Ribeiro *et al*., 1990). *Bixa orellana* is a tropical shrub, native to south American countries and its effective cultivation is reported in many parts of the world (Wealth of India, 1990; Akshatha *et al*., 2011). Historical evidences indicate its extensive distribution and cultivation initially an American tropic and subsequently its spread to rest of the world (Leal *et al*., 2010). Its seeds are composed of an "inner seed" with a shelled kernel containing oils, waxy substances, mineral ash and alkaloid compounds, a peel comprised of cellulose and tannins, and outer cover containing pigments, moisture and a small amount of oil (Ribeiro *et al*., 1990). Bixin, an apocarotenoid devoid of pro-vitamin A activity, is the main oil soluble pigment found in annatto (McKeown *et al*., 1962).

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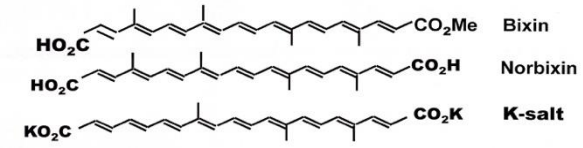
Hydrolysis of the bixin methyl ester group yields the dicarbonxylic acid, norbixin, which is an annatto pigment soluble in aqueous alkaline solutions (McKeown *et al*., 1962; Reith *et al*., 1971). Annatto has been applied to the production of various foods particularly the oil-soluble annatto color is used in dairy and fat-based products like butter, margarine, cheese, baked and snack food (Coulson *et al*., 1980) and also in pharmacy, dyeing of leather and cosmetics (Scotter *et al*., 1998). Annatto color imparts yellow to red with varied hue index as it possess high tincture value, hence have significance in the food industry as a natural food grade color, and stands second in rank among economically important natural food colorants (FAO, 1995, Cannon *et al*.,), apart from its wide use in some regions of the world for non-food applications viz, to color textiles (Aparnathi *et al*., 1980), fabrics and weapons (Rao *et al*., 2002). Earlier review article on Bixa provided a brief information about annatto chemistry (Preston *et al*., 1980), its extraction methods and formulations (Aparnathi *et al*., 1991), pharmacology (Srivastava *et al*., 1999), its toxicology and processing (Satyanarayana, 2003), and methods to analyze its color (Scotter *et al*., 2009). The quality of seed and their geographical condition too had influence on annatto dye yield as evident from various reports wherein, the seeds from Peru are the best with 3-4% bixin content.

The chemical structural formula of the major color pigment in bixa seed that is bixin is as follows-

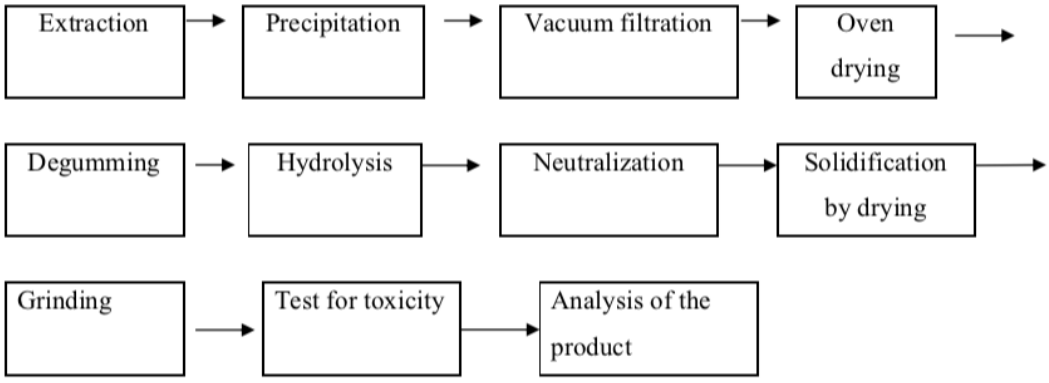


We need both water soluble and edible oil soluble forms of food colors. Again, direct use of natural colorants sometimes causes health problems due to the presence of undesirable compounds in raw dye naturally. Accordingly, the major-colored components on the seed coat that is bixin was extracted, purified and converted into solid form. This is oil soluble form of color. To obtain water soluble solid form of colorant, the alkaline aqueous extract was precipitated, purified, hydrolyzed and then converted into solid form. All these methodologies were developed are most simple, economical and health friendly procedures. The toxicity and the presence of toxic metals were investigated by authorized agencies. Toxicity of the final products were investigated in BCSIR Laboratories, Dhaka and the toxic metals were investigated institute of food safety at Bangladesh Agricultural University, Mymenshingh

All the technologies developed utilize successfully a unique, easily cultivable raw material that is *Bixa* seed. This type of food colorant are crying need of this age for food safety and security.

Materials and Methods

The extraction of annatto color from the seeds of the fruit of the *Bixa orellana* L. tree is done in one or more of approved, food grade materials which includes various solvents, edible vegetable oils and fats, as well as alkaline aqueous and alcohol solutions. Health friendly economic natural food colors are crying socio-economic need for us at the present time. Our prime objective was to develop a simple, economically sustainable and health friendly technology for production of different form of food grade color from Bixa seed. To fulfill above objectives, we avoided organic solvents, any hard treatment by any harmful chemical as per as possible during development of our technologies. The total production methodology can be divided into following steps:



Extraction of color pigment

Color pigment were extracted from annatto seed in aqueous medium at suitable high P^H and at moderate temperature. Moderate abrasion can apply. The major difficulty of such extraction is emulsification. Once emulsion formed, it is difficult to break it to get the bixin and nor-Bixin precipitate. For smooth extraction by aqueous medium the concentration of alkaline agent, time of extraction, P^H of the medium, temperature and even the extent of abrasion are important. For smooth and effective extraction, the optimum conditions are as the Table 1.

Table 1. Optimum conditions for effective extraction of Bixin from raw seed

Concentration of alkali	Temp.	Vol. of water	Time of extraction	Filtration
0.5-1 M	70-80^0 C	5 times of seed wt.	1-1.2 h	Rapid

Precipitation

Precipitation is carried out by decreasing P^H of the extract by the addition of mineral acid solution. In this step also emulsification is a major obstacle. To prevent emulsification, and for smooth and complete precipitation, stirring and concentration of acid are also important. For smooth and complete precipitation of bixin the optimum conditions are as Table 2.

Table 2. Optimum Conditions for complete precipitation of Bixin from alkaline extract

Conc. of mineral acid	Temp.	Stirring	Coagulation temp. & time	Filtration
1 N	50-60^0 C	Moderate	80-90^0 C, 1 h	Vacuum filtration

Degumming

After oven drying of the precipitate at 60-70^0 C for 6 hours the precipitate was de-gummed by stirring magnetically in non-polar solvent like n-hexane for 4 hours followed by filtration.

Hydrolysis

Hydrolysis of the precipitated Bixin to sodium or potassium salt is carried out by calculated amount of alkali as 1.5 Normal aqueous solution at a temperature of 80-85 ^{o}C for 4 hours. This results a dark red colored solution and pH is within 8-8.5.

Neutralization

Sometimes the P^H of the solution obtained after hydrolysis become higher then 8.5. If so happened, the PH may be decreased to the required range by addition of requisite amount of mineral acid. This solution form maybe preserved for 1 year or more.

Solidification

The aqueous solution may be used as liquid form or can be solidified for higher stability and easy handling and transportation to consumers. The aqueous solution was solidified by oven drying at 70-80 ^{o}C for 10 hours.

Grinding

The next step is grinding in a suitable grinder and then sizing.

Toxicity test

The solid product was tested for any toxicity in recognized institute. No toxicity was found.

Test for toxic metal

The presence of toxic metal in solid sample is estimated by atomic absorption spectrophotometric method and compared with permissible limit for human being. The presence of toxic metals are far below the permissible limit.

Results and Discussion

Annatto or Bixin dye is reddish orange in color and imparts a yellow color in water but does not dissolve in it. The principal pigment in annatto extract is bixin, which is contained in the resinous coating of the seed itself. Annatto colors can be extracted from seed coat by various Organic solvents, edible vegetable oils and fats, alkaline aqueous and alcoholic solutions (Marmion *et al*., 2009). But for economic, Commercial and health ground, aqueous medium is the most suitable one. In practice we need solid form of the dye as well as soluble form. We also need water soluble and oil soluble solid form for use in different kinds of food items such as water based and oil-based food items. On the basis of numerous trials an economics, simple and health friendly technology for water soluble food grade solid form of annatto color was developed. This solid form has about two years of shelf life and can be used as food additive to many water-based food items after simply dissolving the color in water. This solid form is tested for toxicity. No toxicity was found in the color samples. This testing report is presented in the Table 3.

Table 3. Toxicity test result of solid food color

Sl. no.	Test parameters	results
1.	Body weight	Unchanged
2.	Mortality	Nil
3.	Activity	Normal activity observed
4.	Diarrhea	Not observed

The presence of toxic metal in solid sample is estimated by atomic absorption spectrophotometric method. The presence of all toxic metals is within the tolerable limit for human being. The results are presented in the following Table 4.

Table 4. Analysis for presence of toxic metals in food color by AAS

Sl. no.	Test parameters	results
As	Not detectable range	6.00
Cd	0.000077	0.003
Cr	0.000305	0.060
Cu	0.00085	0.050
Mn	0.000908	0.050
Ni	0.000298	0.050
Pb	0.00147	0.001
Zn	0.007231	5-15

The color pigment on the seed coat of bixa seed is accompanied with resinous compounds and a few of the organic compounds. The seed also consists some oil. Due to presence of these compounds, there are much possibility of emulsification during aqueous extraction of bixin from seed. This difficulty can be avoided by precious controlling of temperature, total volume of alkali solution, concentration of alkali and stirring time during extraction. If emulsification occurred, it is difficult to break it and the next step that is precipitation is difficult and very small amount of precipitate is obtained. The nor-bixin precipitate is always accompanied by some low polar organic compounds. These impurities can be removed by extracting with low polar organic solvent like n-hexane. This is sometimes called deguming. The purification of bixin precipitate enables it to convert smoothly into dimetallic salt by precise hydrolysis. Nor bixin obtained by precipitation is not soluble in water. For application in most of our water-based food items we need water soluble form of annatto color that is dimetallic salt of nor-bixin. Nor bixin is converted in to water soluble di sodium salt by careful hydrolysis at 70^0 to 80 ^oC. During this hydrolysis the resulting solution must be neutral or slightly alkaline. In this step carefully calculated amount of alkali is added for hydrolysis on the basis of molecular weight of nor bixin. Then the resulting solution is dried very slowly at a temperature of 70 ^oC to 80 ^oC. Thus, crystalline solid water-soluble annatto food color is obtained. If the pH of solution obtained by hydrolysis become much higher, neutralization by calculated amount of dilute mineral acid may be needed.

UV Spectra, perkin Elmer UV Winlab data processor Verwer version-1,01,00, of bixin was recorded and IR spectra, Perkin Elmer Expectrum version-1044, of bixin nor-bixin and sodium salt of norbixin were also recorded. Almost al the absorption signals in these spectra were expected and satisfactory to identify these compounds and the functional groups present in them.

Conclusion

Annatto dye is a natural food color. It does not create any health problem to human being. Our market is flooded with huge imported synthetic food color and create serious and complicated long term health problems. Introduction of natural food colors like annatto food color will save the people from destructive and long-term health problems. In the mean time we developed economically sustainable and health friendly technologies for different forms of food grade color from Bixa seed. Country like Bangladesh is very suitable place for plantation of bixa tree economically. So, we can get huge amount of bixa seed annually from such plantation in unproductive lands at any part of Bangladesh. The chemicals used for the post-harvest processing of bixa seed by our developed technologies to get food grade color is very cheap and nontoxic. So, by a simple technology we can produce huge amount of food grade color from bixa seed without expending any foreign currency. Again, natural food grade colors are demand of the age and indispensable for saving common people from destructive action of synthetic food color available in the market. Finally, since total raw material, chemicals and technologies are our own so we can expect very surely to supply these food colors in cheapest price to common people.

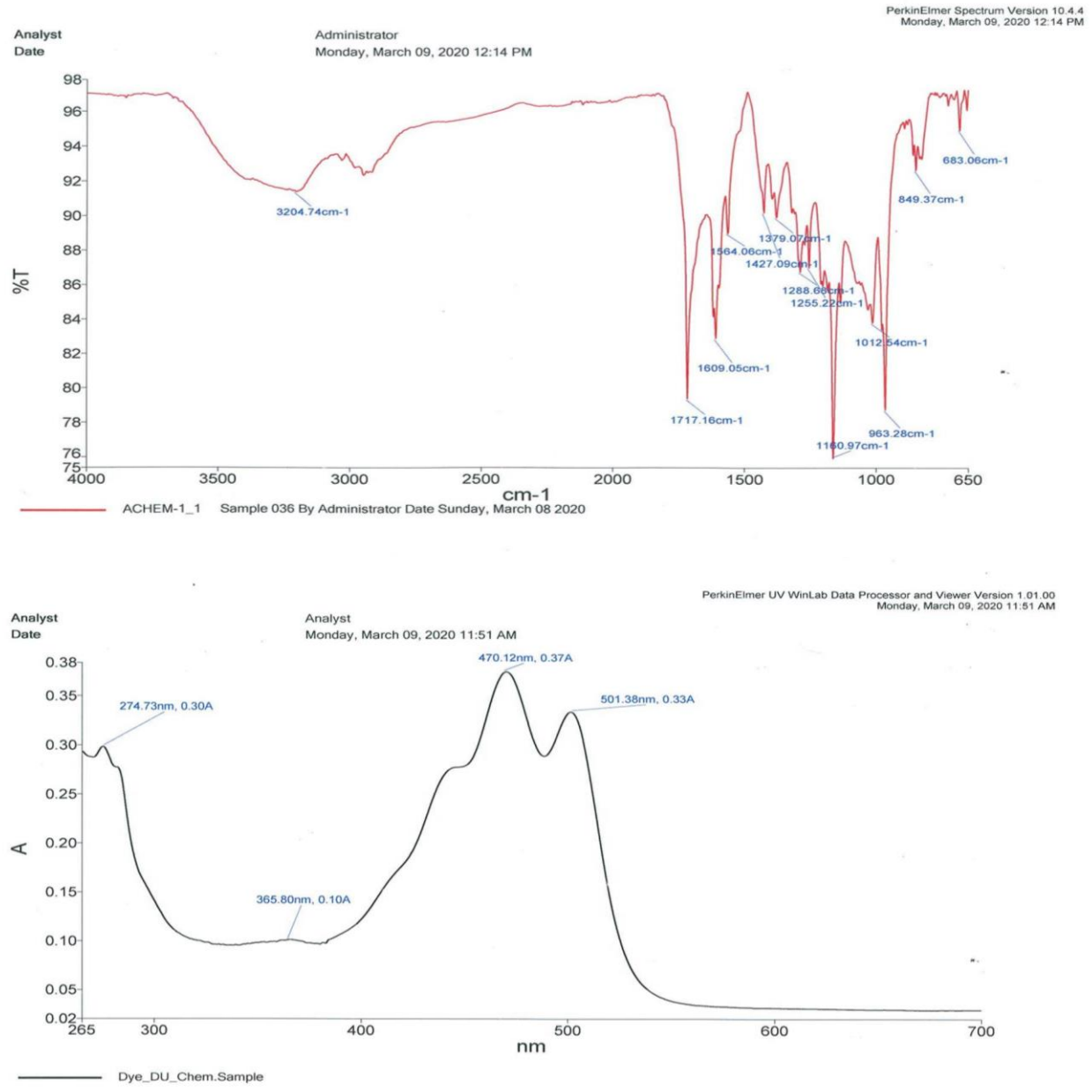
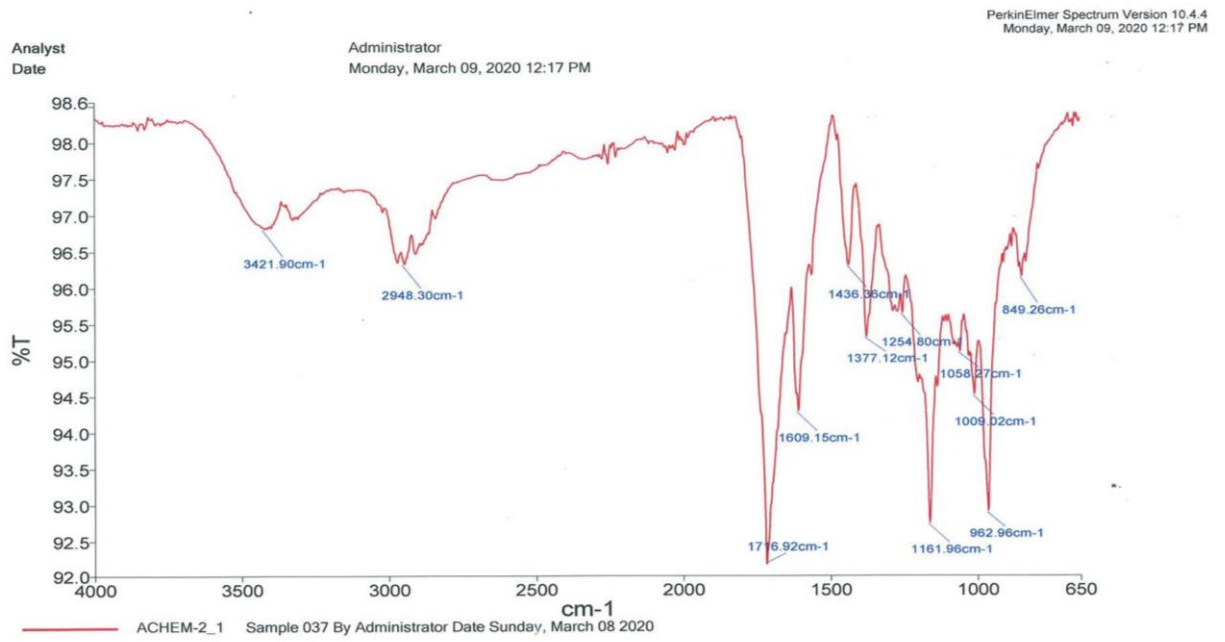
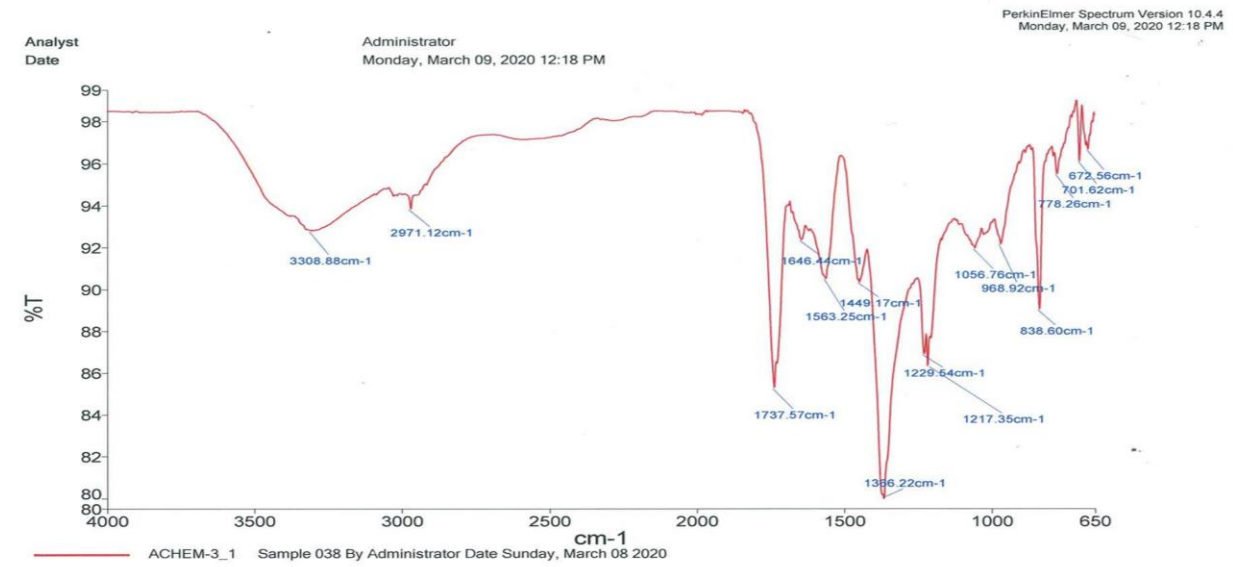


Figure 1. UV and IR Spectra of Bixin



Nor Bixin



Salt of Nor Bixin

Figure 2. IR Spectra of Nor Bixin and Metallic Salt of Bixin

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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