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Review

Bixa Orellana (Annatto Bixa): A Review on Use, Structure, Extraction Methods and Analysis

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Abstract: Safety, environmental and sustainability considerations led to an increasing effort of natural dye to Synthetic dyes. One promising class of alternative natural colorants is the natural annatto dye. Annatto dye is obtained from the thin resinous aril portion of seeds of the *Bixa orellana* tropical plant of great agro-industrial interest and in the food industry given its availability, affordability, and safety. It is also widely used in cosmetics, pharmacy, and dyeing purposes. Bixin and norbixin are the main components of annatto colour which imparts red to yellow hue to the food matrix. Potentially viable methods of annatto dye extraction, extraction yield improvement, its stability in food products, must be employed for better economic prospects. Therefore, this review looks at the potential of isolating and applying annatto from a sustainable source as a possible replacement for synthetic dyes. Extraction techniques, as well as analytical analysis methods, are then discussed. The literature review shows that natural annatto dye had a sustainability impact, was safer for the environment than many synthetic dyes, and their analysis and extraction are simple and fast, making them potential substitutes for synthetic dyes.

Keywords: annatto; bixin; natural dye; norbixin, extraction.

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1. Introduction

By far the most extensively used colorants in the industry are synthetic dyes. However, environmental and sustainability considerations have led to increasing efforts to substitute them with safer and more sustainable natural colorants [1]. In developed countries, the use of artificial colorants like tartrazine (E102), allura red (E129), or sunset yellow FCF (E110) in food products has been heavily questioned, as there have been reports linking indiscriminate consumption of these colorants to the development of degenerative illnesses like cancer [2]. In the United States and Europe, artificial food colors such as carmoisine (E122) and Ponceau 4R (E124) have been restricted, while natural colorants such as the dye obtained from the surface of *Bixa orellana* L. (E 160b, annatto extract) have been suggested [3]. Natural dyes can be classified based on the dye source, application method, and chemical structure. However, because the chemical structure uniquely identifies colors belonging to a certain chemical group with specified qualities, classification based on the chemical structure may be more appropriate [4, 5]. Accordingly, natural dyes can be further classified as indigoids, pyridine-based, carotenoids, quinoids, flavonoids, betalains, tannins, and dihydropyrans [6, 4]. Carotenoids, flavonoids, and quinones are the most common natural dyes [7].

Bixa orellana L. is an ancestral multipurpose plant known as Achiote or lipstick tree due to the reddish-orange dye on its seeds, which was used widely to color the bodies and lips of Central and South America. *Bixa orellana* is a South and Central American native plant that can also be grown in Peru, Mexico, Ecuador, Indonesia, India, Kenya, and East Africa [8]. Plant-derived dyes, insect/animal-derived dyes, and mineral-derived dyes are more biodegradable and environmentally friendly [9, 10].

The seeds of the annatto plant produce one of the most commonly used dyes in the world, not just in food but also in textiles, paint, and cosmetics. Its use has been boosted by the World Health Organization's (WHO) ban on the use of synthetic colors in food and cosmetics, where it is one of the few recommended by the WHO since, in addition to being nontoxic, it does not appear to change the nutritional value of food [11]. Another remarkable fact is that the carotenoid bixin (9′Z-6,6′-diapocarotene-6,6′-dioate) accounts for 70-80% of all natural coloring compounds used in food, cosmetics, and textile dyes [12-14].

Annatto first spread within the type of food colour, conjointly called paprika, a flavoring wide utilized in cookery to reinforce the colour of food. Today, however, its use has spread into several segments of commercial production (food, chemical, cosmetic and pharmaceutical industries) [15] on the skin within the type of makeup and sunscreen, for biological activity and health advantages [16].

In this work, the extraction method and compounds isolated from *Bixa orellana* were searched using the database of different journals, and books. The data were updated in April 2014, using “*Bixa orellana*, chemical, and bixin” as keywords for this review. The references found in the survey were later consulted for details about the models or mechanisms of bioassays used to test the extracts of *Bixa orellana*.

2. Botanical

The annatto tree belongs to the family Bixaceae and the genus Bixa. Despite the existence of several species, the most common in the world is *Bixa orellana* L., named after Francisco Orellana, who was the first European to navigate the Amazon [17]. It is a commercially important plant grown for its natural dye annatto, which is derived from the arils of seeds. The seeds pericarp (the layer that surrounds the seeds) contains colors with wide industrial applications and had a commercial interest. The seeds of annatto constituted "inner seed" with a shelled kernel containing oils, waxy substances, mineral ash, and alkaloid compounds, a peel made up of cellulose and tannins, and an outer cover containing pigments, moisture, and a small number of oils [18].

3. Use and Industrial Application of Annatto

Annatto extract is now one of the more commonly used natural colors in the food, cosmetics, and pharmaceutical industries as it does not alter flavor and is practically non-toxic [19]. It is widely used in the food industry as a natural colorant in many food formulations, including ice cream, cheese, sausages, yogurt, and margarine.

It is also used by the pharmaceutical and cosmetic industries due to its rich source of carotenoids [20]. The therapeutic properties of annatto such as antioxidant and hypoglycemic have been attributed to its high levels of carotenoids [21] Tea made from young roots is used as an aphrodisiac, astringent, and to treat skin issues, fever, diarrhea, and hepatitis by the Piura tribe of the Amazon Rainforest. Annatto is used as antivenin for snakebites in Colombia, and the seeds are used as an expectorant to cure gonorrhea [22].

Annatto has been used as a spice in addition to imparting color to various items. Ground bixa seeds were often utilized in Native American cacao beverages to produce a slight red hue and a musky flavor comparable to paprika or saffron [23, 24]. The usage and amount of annatto in sausages, fish, margarine, snacks, dressings, sauces, and confections differs from country to country due to different food cultures and laws [25, 26].

Table 1. Traditional uses of annatto in different countries.

Country	Use	Parts used	Source
Brazil	Bodypaint, Antipyretic, laxatives/burns, malaria	Seed	[27]
	Antipyretic/laxatives/burns	Seed	[28]
Colombia	Antivenin for snakebites	Seed	[22]
	gonorrhea	Leaves	[22]
Guatemala	Gonorrhea/dysentery, Hepatitis	Leaves	[29]
	Blood diseases	Roots	[30]
	Diabetes	Roots	[31]
Honduras	Aromatic/food coloring	Seeds	[32]
	Pain/digestive/dysentery	Leaves	[33]
Jamaica	Diabetes	Seeds	[34]
	Antipyretic/skin problems	Leaves	[29]
	Alcoholic hepatitis/worms	Roots	[29]
	Antipyretic aphrodisiac, dysentery, astringent, stomach	Seeds	[35]

4. Chemical Compounds and Composition

Bixin accounts for more than 80% of the total pigment content in the annatto seed coat [36]. Bixin was initially isolated from *Bixa orellana* seeds in 1875, and its entire chemical structure and stereochemistry were characterized by 1H and 13C-NMR in 1961. Bixin is an oil-soluble diapocarotenoid with two carboxylic acid groups, one of which is esterified, and nor-bixin is produced by hydrolysis of the ester group in bixin. The oxidative breakdown of C40 carotenoids produces both of these colors [37]. Bixin is a 25-carbon molecule with the chemical formula C25H30O4 (MW = 394.51).

The carotenoid bixin and norbixin pigments are the principal pigments found in anode seeds [38]. Annatto also contains a combination of b-carotene, cryptoxanthin, luten, zeaxanthin, and methylbixin in addition to bixin and norbixin [21]. Long-term trends are a highly unsaturated chemical with chromophores in its conjugated double bonds. Norbixin acts as a sodium or potassium salt in alkali, is water-soluble, and has a significant tinctorial value in water-based formulations. In practice, bixin and norbixin show better light stability than many other carotenoids, but they are both unstable in the presence of air oxygen, just like other antioxidant carotenoids. Relative to other carotenoids, bixin and norbixin have good heat stability during food processing [26].

Bixa orellana seeds, are high in carotenoids, particularly apocarotenes such as bixin, isobixin, and norbixin [39]. Other compounds previously stated for this plant, such as geranylgeraniol, -tocotrienol, -tocotrienol, and eicosatrienoic acid, were also identified [40]. Geranylgeraniol, a key oily ingredient of annatto seeds, accounts for 1% of dry seeds [22]. Other carotenoids present in minor proportions are β-carotene, cryptoxanthin, lutein, zeaxanthin, methylbixin, six apocarotenoids (C30 and C32), eight diapocarotenoids (C19, C22, C24, and C25), and a carotenoid derivative (C14). Furthermore, *Bixa orellana* has been described as the vegetable source with the highest concentration of terpenes like E-geranylgeraniol, which can account for up to 57 percent of total terpenes in the dry seed. Farnesylacetone, geranylgeranyl octadecanoic, and geranylgeranyl formate are some of the other isoprenoids that have been identified. It also contains lipids (linoleic acid, -linolenic and oleic acids in smaller proportions), amino acids (glutamate, aspartate, leucine), and phosphorus, iron, and zinc. Bixin is currently the second most widely used natural color in the world [41-43].

More than 100 volatile compounds have been detected in aqueous and organic extracts of annatto in which 50 of them identified (e.g., bornyl acetate, ∝-caryophyllene, copaene, ∝-cubebene,

(+)-cyclosativene, geranyl phenylacetate, 1-heptanetiol, 3-methylpyridine, 4-methylpyridine γ-elemene, β-humulene, isoledene, β-pinene, seline-6-en-4-ol, δ-selinene, (−)-spathulenol, and (+)-ylangene) [44]. In other study [45] and [46] isolated eight apocarotenoids from annatto seeds: methyl (7Z, 9Z, 9′Z)-apo-6′-lycopenoate, methyl (9Z)-apo-8′-lycopenoate, methyl 1(all-E)-apo-8′-lycopenoate, methyl (all-E)-8-apo-beta-carotene-8′-oate, methyl (all-E)-apo-6′-lycopenoate, 6-geranylgeranyl-8′- methyl-6,8′diapocaroten-6-8′dioate, 6′-geranylgeranyl-6′-methyl-(9Z)-6,6′-diapocaroten-6-6′-dioate, and 6-geranylgeranyl-6′-methyl-6-6′-diapocaroten-6-6′-dioate.

5. Extraction Method

The extraction of annatto is influenced by factors such as the extraction method, type of solvent used, amount of solvent (s) used, solid-liquid (S/L) ratio extraction time and temperature, selectivity of the target component, amount of sample used, extraction efficiency, and the nature and condition of the matrix, just as it is with other plant chemicals. The nature of the solvents is the most crucial of these to dictates the target compound's solubility and extraction efficiency. Depending on the kind of extraction, as well as the solvent and temperature utilized, water-soluble colorants (norbixin) and oil-soluble colorants (bixin) can be obtained [19].

The pigment is extracted from dried annatto seeds using three different commercial methods. These are (i) oil extraction, (ii) organic solvent extraction, and (iii) aqueous alkali extraction [36]. Extraction with oil or solvent primarily yields a colorant called bixin [47]. There are three basic methods for solvent extraction: alkaline extraction (NaOH or KOH solutions), which converts bixin to norbixin; oil extraction (soybean, corn); and organic solvent extraction (hexane, chloroform, ethanol, acetone, ethyl acetate, or propylene glycol), which yields the purest form of pigments. The use of various organic solvents such as ethanol, chloroform, chlorinated hydrocarbons, acetone, ethyl acetate, hexane, methanol, or alcoholic sodium hydroxide, etc., either alone or in combinations at different concentrations or ratios for efficient extraction has been shown in recent research updates, and they are further concentrated by removing the solvents. Various scientific groups have studied various solvent systems and their combinations to improve extraction efficiency. For instance, [48] had used ethyl acetate, acetone, alcohol, chloroform, and 1, 2,-dichloroethane. In another study, [49] used a mixture of solvents like ethanol and chloroform (75:25, v/v) for extraction of the aril of annatto seed.

Bixin is a non-polar chemical with a strong affinity for polar solvents like acetone, ethyl acetate, methanol, and ethanol. According to [39] ethyl acetate has the best solubility efficiency for extracting bixin from annatto seed. Increasing the bixin yield and minimizing contamination by sub-products that affect the extract composition, which harms the stability and power of coloring are both important considerations in the bixin extraction [26]. Solvent extraction is referred to as an indirect extraction approach. The vegetal matrix has a complex microstructure. Because it is made up of cells, intracellular and capillary gaps, and pores, the molecular structure, size, and shape have an impact on the extraction [50]. The low-pressure solvent extraction technique is used in the chemical industry and is also called leaching, decoction, or elution [51]. Mechanical operations, such as grinding the seeds, and physical-chemical methods, such as utilizing solvents or enzymes, can be used to extract the colors in annatto seeds [52].

The methyl group on bixin is saponified by extraction with aqueous alkali (the form employed in the dairy sector, for example, for cheese color), yielding norbixin as the primary colorant [53; 47]. Microcrystalline bixin concentrations with a purity of 80-97 percent can be obtainable and used as annatto concentrates. The solvent extraction approach allows for such high concentrations. Bixin content can range from 1.0 to 6.0 percent, depending on the cultivar and the environment in which it is cultivated; from 1 and 4%, depending on the quality of the seeds [1]. The percentage of bixin is an important factor for commercialization in international markets in which different authors reported the minimum bixin percentage. For instance, ≥ 2.7% ([42]; ≥ 4% [54]; 2.99-5.20% [55] bixin for their commercialization in international markets.

Studies of Annatto pigment extraction have been carried out using supercritical CO2 [56-58] and CO_2 modified with several mixed chemicals (methanol, chloroform, and acetonitrile) [59]. It was shown that the entertainers or mixed chemicals increased the efficiency of extraction. The use of supercritical CO_2 at various pressures and temperatures to extract natural food colors from annatto seeds was found to be more effective than conventional methods for extracting constituents from complex food systems. This could be owing to minimal heat effects on products, high product quality, low energy requirements for solvent recovery, and high separation selectivity [57] microwave-assisted extraction [60-62].

Two extraction methods are known as Pressurized Liquid Extraction (PLE) and Low-Pressure Solvent Extraction (LPSE) in terms of temperature, pressure, solvent to feed ratio, with and without sonication, and solvent type was investigated by [38]. Ultimately, the LPSE method was selected for the extraction time of 85 min with a resulting yield of 4 g/kg at 323 K and 6 g/kg at 333 K. PLE using ethanol as the solvent produced the highest yield of bixin per mass of seeds.

In other cases, advances in extraction with the help of Microwave have led to the development of techniques such as microwave hydrodistillation (MWHD), vacuum microwave hydrodistillation (VMHD), microwave-assisted hydraulic disturbance (MHG), microwave-assisted extraction (MAE), and solvent-free microwave extraction (SFME) [63]. Supercritical fluid extraction of bixin was undertaken by using CO_2 and CO_2 modified with 5% of ethanol as solvents [64]. At atmospheric pressure, the solid-liquid extraction process is carried out, getting the solvent into contact with the substance to be separated. The solvent is removed after extraction, and the extract is concentrated [65; 38; 66]. However, to compete in today's market, pigment-rich product production processes must be not only efficient but also reasonably inexpensive. Spectrophotometry and HPLC methods were used to study methods for extracting and determining annatto in margarine, cheese, and boiled sweets [67]. Another study [68] described the determination of annatto in cheese by a simple acetone extraction followed by concentration by rotary evaporation.

Table 2. Effect of different extraction methods and processing on bixin content of annatto seeds.

Extraction Method	S/F ratio	Extracting solvent	Extraction Time (min)	Bixin	Source
Supercritical Fluids extraction (SFE)	1	CO_2: 5mol ethanol	50	0.80%	[64]
Spouted bed	191	air	240	3.48 mg/100g	[69]
Soxhlet	1	Acetone	480	1.69%	[70]
Leaching	3.1	acetone	60	nd	[43]
UAE (HPLC coupled with diode array and mass Spectrometric	7.3	A mixture of methanol, water acetone	75	1.41%	[15]
LPSE-UAE	8			3.54-7.58%	[38]

6. Analysis of Annatto

Depending on the extraction process and temperature utilized, annatto extracts may contain varying quantities of bixin and norbixin. The tone and colour of the colored food product are determined by the different proportions of these constituents [19]. After the pigment has been extracted, purified, and concentrated, TLC analysis of food colorants for bixin and norbixin must be performed to validate the existence of the pigments extracted. High-pressure liquid chromatography (HPLC), UV-VIS spectrophotometry (UV-VIS), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry can all be used to identify and quantify bixin and norbixin.

6.1. UV-Visible Spectrometry

The UV-Visible spectroscopy analysis of annatto has been well investigated and reported [71-73]. Historically, chloroform and dilute sodium hydroxide (ca. 0.1M) have been utilized as solvents for spectrophotometric measurement of bixin and norbixin, respectively. Bixin has absorption peaks of 470 and 500 nm.

6.2. HPLC systems

An HPLC method was developed from the procedure described by [67] which allowed Chromatographic separation of both isomers of bixin and norbixin. This was achieved by employing a mobile phase consisting of aqueous acetic acid (as an ion-suppressor) modified with acetonitrile. While several published articles on carotenoid analysis by normal phase HPLC are available [74] the vast majority of HPLC methods for carotenoids are carried out by reversed-phase (RP) utilizing a wide range of bonded-phase chemistries. The commonly used stationary phases are those with C18 (ODS) bonded chains, the performances of which are influenced by the extent of end-capping and the carbon loading. Early methods on HPLC analysis of annatto extract [75, 67] reported the use of an isocratic reverse-phase system employing an ODS column and methanol/aqueous acetic acid mobile phase wherein the cis- and trans-isomers of both bixin and norbixin were separated within 10 minutes.

The analytical HPLC-photodiode array (PDA) method developed by [76] provided superior qualitative and quantitative data compared with UV-VIS spectroscopic methods [71, 67]. The colour content (as bixin and norbixin) in 21 commercial annatto formulations was studied through an HPLC-photodiode array [76, 77], particularly concerning the colored thermal degradation products. Although several methodologies have been reported for the HPLC analysis of annatto pigment they don’t necessarily have to give the same result always. The elution time for bixin and nor-bixin may vary depending upon the column type, length, diameter, ratio of the solvent system, brand of the instrument, etc.

6.3. Mass Spectrometry

Like other carotenoids, the MS spectra of bixin and norbixin are characterized by fragmentation leading to losses of toluene and xylene from the polyene chain and the structural significance of the intensity ratio of the ions related to the number of conjugated double bonds. Solid probe electron ionization (EI+) was used to confirm the structures of isolated and purified bixin and norbixin isomers [76]. In another study, the structure of the bixin family of apocarotenoids was determined by EI+ and fast atom bombardment (FAB) MS [78]. Electrospray ionization (EI) and high resolution (HR) matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry was also used to investigate the structure of bixin [79].

6.4. GC Analysis

The existence of principal aromatic hydrocarbon thermal degradation products in annatto color formulations was investigated using an ambient alkaline hydrolysis method followed by solvent extraction and gas chromatography (GC) [80]. GC-MS is also used to analyze the volatile compounds present in water and oil-soluble annatto extracts [81]. The lipid fraction of annatto seeds has been analyzed by GC-MS and they showed the presence of tocotrienols, mainly δ-tocotrienol, but no tocopherols [82] it's chemical composition [83]. Thirty-five components were identified in Bixa seed oil of which the major ones are farnesyl acetate, occidental acetate, spathulenol, and dishware. GC-MS has also been used to analyze the presence of bixin, norbixin, and geranylgeraniol in supercritical CO2 extracted annatto [57].

6.5. NMR analysis

The earliest application of NMR in the study of bixin stereochemistry used low resolution (40MHz) instrumentation to assign 1 H frequencies and deduce that the cis bond of the methyl analog of ‘natural or α-’ bixin was in the 9′- (equivalent) position [84]. The high-frequency shift of the proton assigned to H-8′was attributed to deshielding by the 11′-12′ alkene bond when compared to the trans- (or β-) isomer, which was confirmed via synthesis and more detailed structural assignments [85]. Similarly, X-ray diffraction in conjunction with NMR and mass spectrometry has been used by a combination of 1D and 2D NMR techniques to determine the structure of the bixin family of apocarotenoids [78]. To elucidate and compare the crystal structures of the cis- and trans-isomers of bixin and methyl bixin, scientists used chemical shift, coupling constants, and 1H correlation data, as well as ion abundances and intensity ratios from standard electron impact (EI+) and fast atom bombardment (FAB+) MS spectra, and bond measurement, cell dimension, and degree of hydrogen bonding from X-ray diffraction data.

7. Conclusions

Bixin and norbixin are the main components of annatto which impart red to yellow color to commercial production (food, chemical, cosmetic and pharmaceutical industries. Potentially viable methods for developments of annatto dye extraction, extract yield improvement, stability of annatto color in food products, medicines, cosmetics are useful for sustainability and replacement for synthetic dyes. Extraction techniques, simple and fast extraction as well as analytical analysis methods used for annatto sustainability impact, safety for the environment make them potential substitutes for synthetic dyes.

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