

A Study on Extraction of Plant Based Bio Colours

P.Vijetha^{1*}, R. Venkata Nadh², K. Ramesh Naidu¹ and T.Swapna Sundari¹

^{1*} School of Chemical Engineering, Vignan's University, Vadlamudi - 522213, India,

²GITAM University - Bengaluru, Karnataka - 561203, India

Abstract- Most of the synthetic colours are carcinogenic. Many natural colours are thought to play- a significant role in preventing or delaying the onset of many diseases. Optimized conditions were arrived for the extraction of plant based biocolours based on the literature survey. In the present study, extraction of bixin from annatto seeds was carried out using ethyl acetate and ethanol. A pure bixin is obtained by precipitating from petroleum ether. A simple method for preparing butter colour from annatto seeds is tested using castor oil and groundnut seed oil. Lycopene was extracted from tomatoes using ethyl acetate as a solvent and further purified by successive recrystallisation from ethyl acetate and ethanol. A stable oleoresin of lycopene was prepared in soybean oil. Purity of extracted colours is established from spectral studies.

Keywords - Anntto seeds; bixin; lycopene; plant based colours; tomato.

I. INTRODUCTION

Colour has been added to our foods in some or other form for centuries. Some of chemical based colours have the potential to damage the nervous system and kidneys and can even stunt the physical and mental growth of children [1]. Now-a-days, natural colours are mainly employed in the food industry. Similarly, at the time of Hindu festival of colours, Holi, doctors issue warnings to potential buyers to avoid cheap synthetic colours flooding the markets, which are toxic, can result in any thing from skin allergies to cancer, eye irritation and blindness [2]. Hence, natural colours are also used in different industrial products viz., pharmaceuticals, textiles, papers, cosmetics, and paints [3]. These applications are helping to increase opportunities in various potential applications like restoring works of art and bio-building industry. The colours with biological origin are called as "Biocolours" and in general they are extracted from different parts of plants (fruits, vegetables, seeds, roots) and microorganisms [4]. Annatto, turmeric, carmine, grape skin extract, caramel, paprika and beet are some of the sources for plant based biocolours.

Bixa Orenella is commonly associated with the annatto dye obtained from the seeds. Its earlier use for dyeing of fabrics has

ceased with the development of synthetic dyes [8] [5]. Bixin (Fig. 2) is the main component and this is at present in considerable demand as a non-toxic fat-soluble food colour [9] [6]. Annatto dye finds its use in Dairy Industry for coloring butter, cheese and margarine. It is also used in ice cream, bakery products and edible oils. Annatto dye is also used by the cosmetic industry in products such as lipsticks and suntan preparations. It is also used for dyeing of leather as an ingredient in floor wax; furniture and shoe polish mail, glass brass lacquer, etc [10] [7]. In India, Annatto (Bixa orellana) is a shrub growing in the forest regions of Visakhapatnam and Srikakulam, Trivandrum, Malabar & Coromandal Coasts, and in certain districts of West Bengal, Maharashtra, Assam and Karnataka [11] [8].

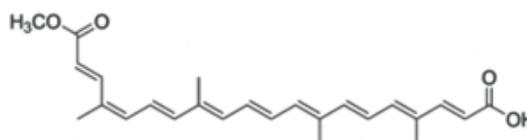


Fig 1. Bixin

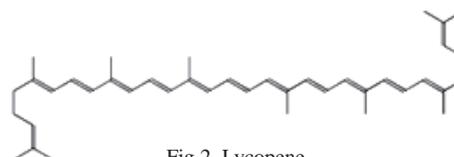


Fig 2. Lycopene

Lycopene is a natural pigment (Fig.1), particularly abundant in tomatoes and watermelon, having intense red colour [5] [9]. The amount of lycopene in a tomato is variable, ranging from 15-56 mg/kg. Lycopene is a strong antioxidant and can help prevent prostate cancer, lung cancer, stomach cancer, and possibly other cancers including bladder, breast, cervix, pancreas, colon and rectum, and oral cavity [6] [10]. Due to its safety and beneficial effects, lycopene is widely used in the food industry as a colouring agent, usually in the form of oleoresin, i.e., a suspension in natural lipids. In this form lycopene is prevented oxidation (crystalline lycopene being highly unstable) and bacterial degradation which is most likely due to the lipids and natural antioxidants present. Furthermore, lycopene is used as food supplement due to its

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Page No : 1

antioxidative and chemo protective properties [7] [11]. However, it is known that tomato farmers are dumping their crop in several parts of India as prices crash. Therefore, commercial extraction of lycopene from those excess produced tomatoes will help the farmers.

In spite of advantages associated with plant based biocolours (e.g., renewable resources, potential to be available in large quantities, low environmental impact, possess biological properties, good range of colours), they are not competitive in terms of cost, supply and standardized quality. Whereas, natural plant based biocolour market is growing on a global scale at a greater rate than synthetic colors owing to a continued consumer pressure to 'go natural' [1]. Hence, an attempt was made to select standardized protocols based on literature survey for the extraction of (a) bixin from annatto seeds followed by preparation of butter colour and (b) extraction of lycopene from tomatoes.

II. EXTRACTION OF BIXIN FROM ANNATTO SEEDS

Extraction of bixin from annatto using a Soxhlet apparatus is slow and found darkening the colour. Therefore applying mechanical pressure using discrete amounts of solvents followed by direct boiling in a flask and decanting the extract through muslin cloth was found to be most convenient [11]. The seeds of bixa orellana (90 gm) were directly boiled with ethyl acetate. Decantation, concentration and filtration resulted 1.0 gm of pure crystalline labile bixin. The filtrate was poured in to excess of petroleum ether with vigorous stirring, when 0.85 grams of a deep red solid was precipitated. Further purified by taking the ethyl acetate solution of the solid and diluting it with petroleum ether, where pure bixin was precipitated (Fig. 3 and 4). The yield of the pure bixin is 0.9%.

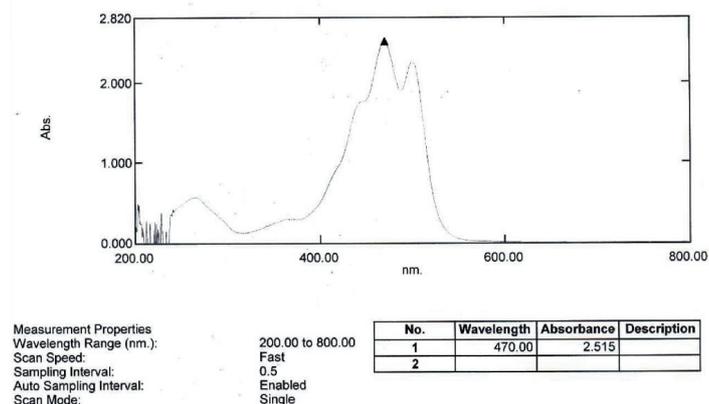


Fig.3. Visible Spectrum of Bixin

Preparation of Butter Colour from Annatto Seeds:

The best standardized extraction process [12] is as follows. The seeds cooked in autoclave (at 12 lb/in²) are mixed with five times their weight of castor oil and extracted in kettles maintained at 120°C for 2hr, keeping the contents continuously stirred. The seeds are then removed by filtration. Uniform shade of butter color is

maintained by diluting with 1000 parts of ethyl alcohol to show 5 yellow units and 0.4 red units in a Lovibond Tintometer with a 1 cm cell [13]. As annatto color is highly susceptible to oxidation when exposed to light and air it deteriorate more rapidly in glass bottles than in steel drums. Therefore, it was recommended to store butter color preferably in a cool place in closed tin or steel containers with as little air space as possible [14].

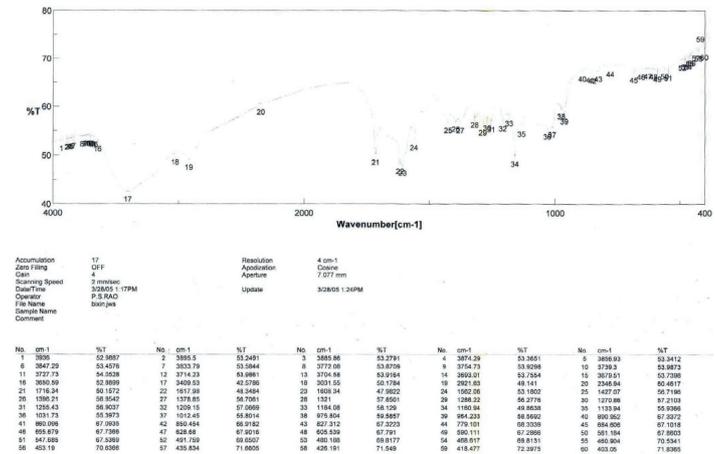


Fig.4. IR Spectrum of Bixin

III. EXTRACTION OF LYCOPENE FROM TOMATO

The major steps involved in the extraction process of lycopene from tomato [15] are given here. (1) pretreating 1 kg fresh tomatoes (washing, cutting / crushing) and (2) heat concentrating (distillation under reduced pressure - 20 mBar at 50°C) to 1/3rd volume (3) extracting the concentrate at room temperature by shielding from light for four times using water-saturated ethyl acetate (twice the weight of the concentrate per each time) (4) backwashing the extract with water (5) concentrating the extract to dryness under reduced pressure (6) after crystallization, crystals were filtered and dried under vacuum at 50°C to obtain 0.75 g of crystalline lycopene (7) oleoresin is prepared by adding soybean seed oil (1.2 g) to the above obtained lycopene crystals (0.75 g) (8) On the other hand, lycopene obtained in step 7 is further purified by suspending in ethanol (10 ml), vacuum filtering, washing with ethyl acetate and vacuum drying at 50°C to obtain high pure crystalline lycopene (0.04 g) (Fig. 5).

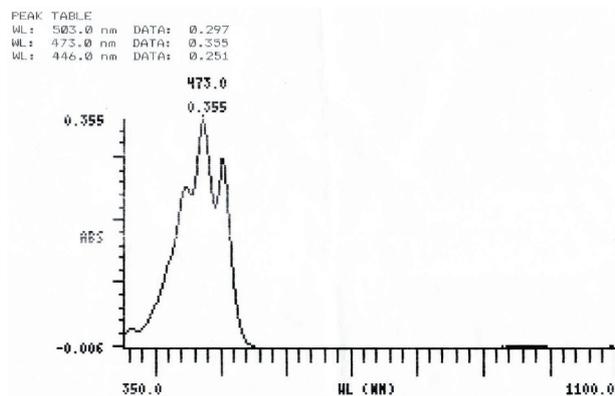


Fig.4.3 Visible Spectrum of Lycopene

IV. CONCLUSION

Polar solvents are preferable compared to non-polar solvents to extract the bixin from annatto seeds. As extraction in a Soxhlet apparatus is slow, darkening the colour was observed, applying mechanical pressure using discrete amounts of solvents followed by direct boiling in a flask was found to be most convenient. Spectral studies indicate that a pure bixin is obtained by precipitating from petroleum ether. For the preparation of butter colour, the extract was found to be more intensified with castor oil compared to groundnut seed oil. Lycopene was extracted from tomatoes using ethyl acetate as a solvent. Further lycopene was purified by recrystallisation from ethyl acetate and ethanol successively. In addition a stable oleoresin of lycopene in soybean oil was prepared.

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