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Extraction of Astaxanthin Pigment from Shrimp Waste for Application in the Textile Industry

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Abstract : A method that is both efficient and good for the environment is proposed for making astaxanthin, a high-value pigment, from shrimp waste, which is a low-value raw material. The goal of this study was to find out how the natural astaxanthin found in shrimp shells could be used in the textile industry. Application of the astaxanthin pigment was evaluated for different grades of textile materials commercially available in the market, which include cotton, pure cotton, polyester, nylon, and woolen. The extraction of red-orange pigment from shrimp waste and its subsequent use in a variety of textiles is the focus of the current research (Cotton, Pure cotton, Polyester, Nylon, and Woolen). Therefore, it is concluded that thiourea is an efficient mordant for processing colored textile materials.

1.INTRODUCTION

In the last several decades, aquaculture has expanded faster than any other kind of food production. Aquaculture is the controlled and commercial farming of aquatic animals with the goal of increasing production while keeping ownership of the stock. This is different from fishing because it makes it easier to increase the number of species needed for food, industry, or fishing for fun. As H. C. Ramrez Saad et al. point out, fishing and aquaculture help India's agriculture a lot. When it comes to expanding aquaculture, India has a lot of untapped potential. The total length of its shoreline is 7,517 kilometers, and its river and canal network is 195,210 kilometers long. This system is made up of 14 large rivers, 44 medium-sized rivers, and a lot of smaller rivers and streams. Furthermore, we estimate that we have access to 2.36 million ha of pond and tank space.

J. De Jong et al., India's Resilience was on display in 2012, when marine fisheries production hit a record high of 4 million tonnes. Even though there are social and economic problems in India, the federal and state governments have come up with good policies and ways to run the country's marine fisheries. Better nutritional needs and protein content is a national aim in India since the country's population is predicted to reach 1.63 billion by 2050, with the younger generation accounting for a larger part of it. At a total farm gate selling value of USD 263.6 billion in 2018, global aquaculture output hit a new all-time high of 114.5 million tonnes of live weight. There were a total of 82.1 million tonnes of aquatic creatures, 32.4 million tonnes of aquatic algae, and 26,000 tonnes of seashells and pearls used for decoration. In 2018, finfish farming was the most popular method for raising aquatic animals. Across the globe, the output of feed aquaculture (57 million tons) has surpassed that of the non-fed sub-sector. The global output of farmed aquatic animals increased by 5.3% year on average between 2001 and 2018, but by only 4.0% in 2017 and 3.2% in 2018. Around 24426.74 crores, or 6.62 percent of the world's aquaculture output, came from India's 140656 hectares of salt-affected coastal areas that can be used for aquaculture in brackish water. P. Ravichandran et al. Inland salt water, the most popular fish species are barramundi, Pangasianodon hypophthalmus, Chanos chanos, Mugil cephalus, Etroplus suratensis, and common carp. India also grows a large number of shellfish species in salt water that is close to land. These include Litopenaeus vannamei and Macrobrachium rosenbergii. The authors Biswal.A.,

1.2 APPLICATIONS OF CRUSTACEAN AND FISH WASTE

Fishing bio-waste is often wasted since there is no uniform recycling technique. Waste happens when things that could hurt the environment are thrown away because of technological or financial limitations. Biochemical can be made from this waste. New waste processing methods are needed to produce high-quality byproducts with minimal environmental impact. This will increase fishing sector jobs and growth. Crabs and bivalves produce beneficial trash. Crab, lobster, and shrimp shells contain chitosan sugar. et al. (2019). Cement, fake stones, air and water purifiers, and walls and ceilings are made from oyster shell waste. Pharma uses it. T. Ghassemi et al. (2021). Chitin and chitosan are plant evokers and elicitors. Crab shell calcium carbonate cleans water. Drugs and medicines use crab shell calcium carbonate (Jamari, J. et al., 2022). Secondary metabolite production boosts plant defenses. Antibacterial and antifungal medicinal agents with applications in agriculture, medicine, the environment, food, cosmetics, and textiles. et al (2020). Antifouling membranes use chitosan. Chitosan nanocomposite coating can keep food fresh, clean water, and make anti-corrosive and self-healing paint. J. Dutta et al. (2019).

1.3 PIGMENTS

Pigments have been used all the way back to ancient times, and they may be found in ores, insects, plants, and even animals. Since the middle of the 1800s, synthetic dyes have mostly replaced natural colors, even though they are bad for people, animals, and the environment. Substituting natural or artificial colorants for synthetic pigments Reference: Usman, H. M., et al., 2017.

1.4 ASTAXANTHIN

Astaxanthin is a xanthophyll carotenoid found in a variety of microbes and marine animals. Although some studies claim that astaxanthin has more potent biological activity than vitamin A, it is a red fat-soluble pigment that does not have pro-Vitamin A activity in the human body. Goycoolea, F. M. et al. (2006).

1.5 ASTAXANTHIN STRUCTURE

The two outer rings of astaxanthin are joined together by a polyene chain. At the 3, 3′ positions of the -ionone ring, there are two carbons that are not the same. The molecule has two hydroxyl groups, one at each end (-OH). Carotenoids' molecular structure, chemical properties, and light-absorbing abilities are all unique because of their polyene system (conjugated double bonds). Two possible geometric isomers, cis and trans, exist for each double polyene link. The most naturally occurring carotenoids are the trans isomers. It has been shown that trans-astaxanthinis more stable than cis-astaxanthin from a thermodynamic standpoint. To cite this paper: Pedrosa, L. F. C., et al. (2010). Although there have been concerns raised about the safety of using syntheticastaxanthin in human consumption, the astaxanthin found in *Haematococcus pluvialis* is used in awide variety of human uses, including nutritional supplements, cosmetics, and even food. Depending on the relative orientation of the hydroxyl groups on the molecule, astaxanthin may exist in various different stereoisomers in nature, including (3S, 3'S), (3R, 3'R), and (3R, 3'S). *Haematococcus pluvialis* and salmon species often carry the stereoisomer 3S, 3'S. Grazia Scapagnini et al (2018). Figure 1.

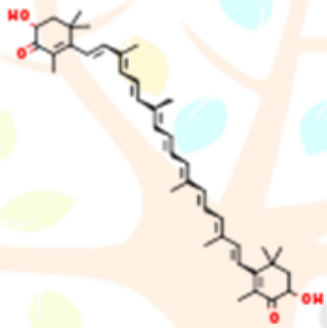


Fig 1. The Structure of Astaxanthin

1.6 APPLICATION OF PIGMENTS IN THE TEXTILE INDUSTRY

Because of their synthetic origin and intricate molecular structures, which restrict their biodegradability, artificial pigments create effluents that represent a severe danger to water supplies and the environment. As a result, the textile industry is now searching for natural pigments. There are several synthetic colors that have been banned because they cause cancer and allergic reactions in people. Even if certain colors have not been made illegal yet, this does not mean that they are completely risk-free. Affat. S.S. et al. (2021). Prodigiosin is a reddish-yellow color that comes from a type of sea bacteria called Serratia sp. It is used as a dye for commercially available fabrics like cotton, chiffon, Poplene, pure silk, Century cotton, Dupoilsilk, Organdi, Polyester, Terry cotton, and nylon. Chandrasekaran, M. et al. (2008). The commercial printing method of using chitosan as both a thickener and a binder in pigment printing was looked into. Chitosan was used in the process (Alcoprint). By putting printing pastes made of chitosan, pigment, and acetic acid in the right amounts on polyester and 67:33 polyester/cotton woven fabrics, high-quality prints were made. When the chitosan print was allowed to cure at a temperature of 150 degrees Celsius for six minutes, the color fastness of the samples was equivalent to that of samples that had been professionally printed when rubbing and washing tests were carried out. Holme, I. et al. (2000), from rotting mango, scientists were able to find a type of fungus that can make colors that can be used to dye fabrics. The decaying mangoes were selected as a food source for a number of different types of fungus. A specific fungal isolate was chosen for further study because it was clear that it made secondary metabolites. The fungus was found to be of the Talaromyces verrucosus strain by using internal transcribed spacer sequencing. So that the harvest could be as big as possible, the growth of the fungus was sped up and their pigment production was made better. Cotton fabric was dyed with the natural pigment after it was removed using a method that is often used for dying fabrics with natural pigments. Reza, M. A. et al. (2017) studied *Scytalidium cuboideum*, a red crystallizing pigment that has shown exceptional textile resistance in the past. Three different ink formulas, one of which included the red pigment, were used to print on cotton and polyester fabrics to see how well the pigment worked in an inkjet setting. These ink formulations were hexadecyltrimethylammonium bromide (CTAB), ethanol, and acetone. Because of the CTAB and ethanol-based ink formulations, the surface of the cotton and polyester fibers produced a structure similar to a mesh, which caused the fabric to become purple when it was dyed.This phenomenon is common in the textile business. (2019)Robinson,S.C.,et al.

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION

The shrimp waste was procured at the kasimedu fish market in the Indian city of Chennai, Tamil Nadu. Astaxanthin is taken from shrimp by putting the telson, carapace, cephalothorax, chelipeds, and perciopods in a sterile ice bath and taking them to a lab. Keep at room temperature (20°C) until ready to use. The garbage was flushed down the toilet a couple of times before it was used. The shrimp byproducts

were then sun-dried for a day. To make the powder, we collected dried shrimp parts (Telson, carapace, cephalothorax, chelipeds, and perciopods), ground them twice to make sure they were properly milled, and sieved them through a 0.2 mm mesh. The powder was then kept in a dry container until usage.

2.2. EXTRACTION OF ASTAXANTHIN PIGMENT

In a conical flask, 20 grams of shrimp waste powder were soaked in 150 milliliters of acetone for four days. The mixture was then filtered through Whatmann filter paper and put in a different conical flask. The acetone in the solution was allowed to evaporate over the course of two days by leaving the petri plate out in the open. After two days, the unrefined pigment was scoured with a spatula and placed in a dark bottle.

2.3 ASTAXANTHIN PIGMENT THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS

Acetone was used for both the stationary and mobile phases, and precoated silica plates were used to separate them. After closing the sterile beaker with a watch glass, the mobile phase was introduced. After incubating the silica plates for 10 minutes at 60ºC in a hot air oven, the excess was trimmed off. Once the silica plates were cool, the astaxanthin pigment was put in each of the marked spots.

We waited for the solvent front to reach 3/4 of the TLC plate before removing the plate from the beaker. After 5 minutes, the plates were taken out of the beaker and dried at room temperature. They were then put in natural light and a UV-chamber, where they measured how far the compound and the total solvent had moved.

2.4 UV –SPECTROPHOTOMETER

1. The absorbance at 535 nm of purified astaxanthin is measured using an ultraviolet-visible scanning spectrometer.
2. The cuvette was filled with 1 ml of acetone and used as a blank. "O" indicates blank.
3. The other cuvette contained 1 ml of the prepared extract and put the cuvette back into the apparatus.
4. Initialize the UV spectrometer's wave length setting.
5. And the machine scanning the present of maximum absorbance in the extract

2.5 TESTING OF PIGMENTS IN DIFFERENT CLOTH MATERIALS

In this study, different types of cotton, pure cotton, polyester, nylon, and wool were used to look at how the pigment astaxanthin could be used in different types of textiles. For the study, each type of fabric was cut into 2 cm2 pieces that were all the same.

2.6 FIRST SET

A magnetic stirrer is used to mix together 5 milliliters of acetone and 1 gram of pigment. Fabric squares measuring 2 by 2 inches were sliced off (cotton, pure cotton, polyester, nylon, woolen). After applying one or two drops of pigment to a warm surface, the fabric was allowed to cure at room temperature for roughly an hour. The fabric was washed in soapy water and then rinsed in cold water before being dried at room temperature for half an hour. Normal and ultraviolet light were able to penetrate the fabric after the wash.

2.6 SECOND SET

After cutting the fabric into 2x2 squares, 1g of pigment is combined with 5ml of acetone (cotton, pure cotton, polyester, nylon, woolen). After applying one or two drops of pigment to thecloth's warm surface and letting it dry at room temperature for one hour, the fabric was submergedin 1% thiourea for 30 minutes at 70 degrees Celsius. After 30 minutes, the cloth was hung up to dry at room temperature after having been washed with the soap solution and regular tap water. After being washed, the fabric absorbed under UV light.

2.7 THRID SET

The fabric is cut into 2x2 squares, and 1 gram of dye is mixed with 5 milliliters of acetone.The dye can be used on cotton, pure cotton, polyester, nylon, or wool. A water bath set at 60^0C was used to incubate the cloth with 1 to 2 drops of pigment for an hour. The fabricwas washed in soapy water and then rinsed in cold water before being dried at room temperature for half an hour. Clothes were soaked up by regular and UV light after washing.

2.8 FOURTH SET

The fabric is divided into 2x2 squares, and 1g of pigment is diluted in 5ml of acetone (cotton, pure cotton, polyester, nylon, woolen). After putting 1–2 drops of pigment on the warm surface and letting it dry at room temperature for 1 hour, the cloth was put in 1% thiourea at 70°C for 30 minutes. After 1 hour in a water bath at $60\ ^0$C, the cloth was washed with soapywater, rinsed in cold water, and dried at room temperature for 30 minutes. Fabrics were able to soak up both regular and UV light after the wash.

3.RESULTS

3.1 TLC ANALYSIS

Thin-layer chromatography was used to look at how many pigments were in the extract, and acetone was the main organic solvent. The stationary phase and the moving phase were madeup of acetone and silica plates, respectively. The beaker that held the mobile phase was cleaned, and the lid was made of watch glass that had been sterilized. After taking out the right amount of silica, the plates were put in a hot air oven at 60 degrees Celsius for 10 minutes. Once the silica plates were cool, the astaxanthin pigment was put in each of the TLC plates' marked spots We waited for the front solvent to reach three-quarters of the TLC plate before removing it from the beaker. After 5 minutes, the plates were taken out of the beaker and dried at room temperature.They were then put in natural light and a UV-chamber to measure how far the compound and thetotal solvent traveled. A pigment's RF value was used to determine its path from origin to destination (Retardation factor). Figure 2.

$$R_f \text{ Value} = \frac{\text{Distance Travelled by the solute}}{\text{Distance Travelled by the total Solvent}}$$

Calculation

$$R_f \text{ Value} = \frac{6.7}{7.5} = 0.85$$

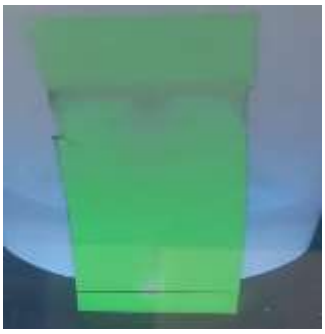
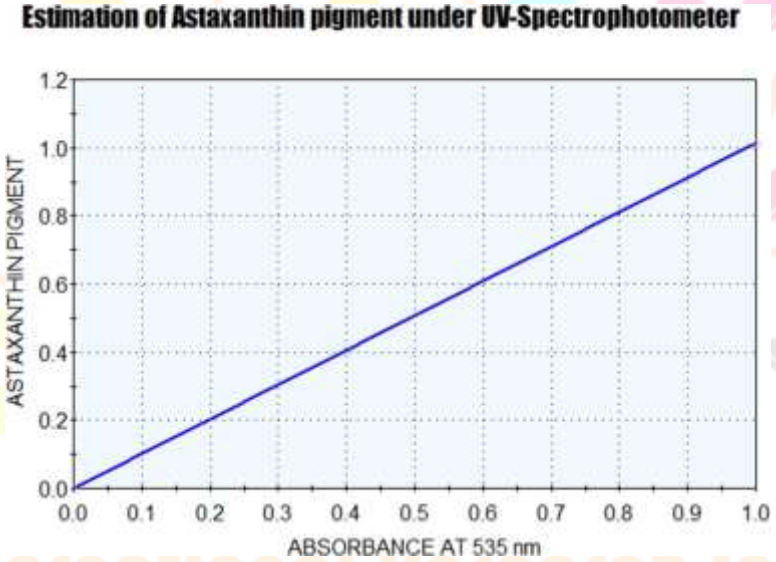


Fig.2.TLC analysis of Astaxanthin pigment

3.2 UV- SPECTROPHOTOMETER

An Ultraviolet-Visible scanning spectrometer is used to measure how much astaxanthin isabsorbed at 535 nm. The cuvette was filled with 1 ml of acetone and used as a blank. "O" signifiesblank. One millilitre of the extract was put in the other cuvette. In this study, the maximum absorbance needed for extraction was found by setting an Ultra Violet-Visible spectrophotometerto a wavelength of 1.013 in Figure3.

Fig.3. Estimation of Astaxanthin pigment under UV-Spectrophotometer



3.3 ANALYSIS OF PIGMENT ABSORBED IN DIFFERENT CLOTHS

Pieces of 2 cm^2 were cut from the fabric. Furthermore, the pigments were applied in four sets, with the first set being the application of one to two drops of pigment to the cloth material ona warm surface and the subsequent washing, rinsing, and drying of the fabric with the soap solutionand tap water. In the second series, 1–2 drops of pigment were applied to the warm surface of thecloth and let to dry at room temperature for 1 hour before being dipped in 1% thiourea for 30 minutes at 70°C. The fabric was then washed with soapy water and hung to dry before being used.Third, we added 1–2 drops of pigment to a clean piece of fabric, placed it in a test tube, and placedthe entire thing in a water bath set at 60° C for 1 hour before washing it with soapy water and letting it air dry. After the fourth set of fabric was dipped in 1% thiourea at 70° C for 30 minutes, the pigment was applied with 1–2 drops onto the warm surface and left to dry at room temperaturefor 1 hour. The sample of fabric was placed in a test tube and heated in a water bath for one hour at 60 degrees Celsius. After 30 minutes, the cloth was hung up to dry at room temperature after having been washed with the soap solution and regular tap water. All of the clothes can be seen in both regular and UV light after they have been washed.

The comparison showed that the third and fourth sets absorbed the pigments the best. Woolen and pure cotton, in particular absorbed more pigment than polyester, cotton, and nylon cloth, particularly in the 3 and 4 set in Figure 4.

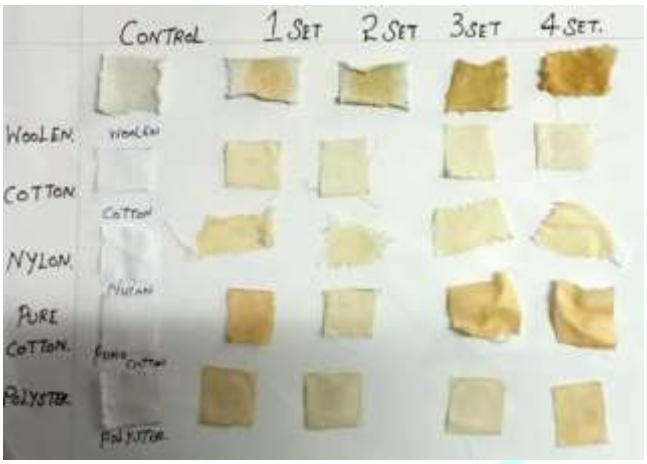


Fig 4. Astaxanthin pigment absorbed in different materials

4. DISCUSSION

In order to obtain a colorless extract from the powdered shrimp waste, 5g of solvent was mixed with (3:1) acetone three times until the solvent was colourless, and then the extract was filtered using whatman paper. After collecting the epiphase and separating it with the separating funnel, the extract was put in a water bath for 50 minutes to get rid of the petroleum ether. Using a UV-visible scanning spectrometer, the absorbance of an extraction was determined between 190and 1000 nm. According to these results, the peak absorption occurs at 466 nm. With an Rf valueof 0.36, the TLC (Thin Layer Chromatography) test proved that astaxanthin was there. As of 2015(Kumaresan, R.).

In this study, the shrimp waste was acquired from a nearby market in Chennai, Tamil Nadu,India. Astaxanthin is extracted from shrimp by freezing their telson, carapace, cephalothorax, chelipeds, and perciopods, then bringing those frozen specimens to a lab on sterile ice. Put it in thefridge at 20 degrees Celsius until needed. The garbage was flushed through a water system beforebeing put to use. The shrimp byproducts were left out in the sun for a day to dry. After collectingdried shrimp waste (Telson, carapace, cephalothorax, chelipeds, and perciopods), milling it twice to make sure it was ground well and sieving it to a mesh size of 0.2 mm, it was stored in a dry place until it was needed. 20gm of shrimp waste powder were added to one hundred fifty millilitresof acetone in a conical flask, allowed to soak for four days, and then filtered through Whatmann filter paper. After letting the acetone evaporate for two days in the air, the solution was poured intothe periplate. After two days, the rough pigment was scraped with a spatula and placed in the darkbottle until used. The combination of acetone and a stationary phase of precoated silica plates wasused. After adding the mobile phase, the sterile beaker was sealed with a sterile watch glass. The necessary silica plates were sliced and then heated in a hot air oven at 110 ^{o}Celsius for 15 minutesto activate them. As soon as the silica plates had cooled, the astaxanthin pigment was placed in each of the TLC areas that had been previously designated. When the solvent front reached three-quarters of the plate, the plates were removed from the TLC chamber. After 5 minutes of drying time in the air, the plates were soaked up by sunlight and ultraviolet light in a UV-chamber. The Rf value of 0.89 confirms the presence of astaxanthin. Prodigiosin, a pigment produced by the bacteria Serratia sp. BTWJ8, has dyeing propertiesand can be used to colour a wide range of textiles (Cotton,Chiffon, Poplene, Puresilk,Century Cotton,Dupoilsilk, Organdi ,Polyester, Terrycotton, and Nylon). In addition, wash performance tests on textile materials treated with pigment and thiourea, a safe and effective mordant, and the cloth examined under the UV-spectrophotometer for 535 nm absorbance, suggest that this pigmenthas considerable potential for application as a dye in the textile industry. According to research (Chandrasekaran, M., 2011)

In this experiment, we took commercially available cloth (cotton, pure cotton, woollen, nylon, and polyester), cut it into uniform sizes, and sprayed 2 to 3 drops of pigment onto each square. There are four distinct palettes used for dying the fabric. In the first set, ten percent of the pigment is absorbed by the cloth, twenty percent in the second, thirty to forty percent in the third and fourth sets, and so on. The greatest absorption rates for dyes are found in pure cotton and wool.Refer Table 1.

Absorption of pigments in the cloth under UV-light	
SETS	**PERCENTAGE**
1ST SET	**10%**
2nd SET	**20%**
3rd SET	**40%**
4th SET	**30%**

Table 1. Absorption of pigment in four Sets

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