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Research Progress of Natural Edible Blue Pigment

Luwen Huang, Liping Zhu*

School of Bioengineering, Qilu University of Technology, Shangdong, China
*zhuliping2012@aliyun.com

Abstract: Natural edible blue pigment is a scarce pigment among natural pigments, mainly derived from animal and plant tissues, microbial fermentation metabolites. Among the plant natural blue pigments, the ones that account for the widest market of edible natural blue pigments are algal blue pigment and gardenia blue pigment. The sources of microbial natural blue pigment include some fungi and bacteria in addition to microalgae, mainly focusing on the study of Streptomyces, Pseudomonas, Pseudoalteromonas, purple non-sulfur bacteria, Dourollerella, and sprouting short-stalked mold. The natural blue pigment is unstable, so it is of far-reaching significance to improve the extraction process, especially the research on the isolation and purification of blue pigment produced by microbial fermentation. High-speed frozen centrifugation, solid-phase extraction, high performance liquid chromatography, nuclear magnetic resonance spectroscopy, mass spectrometry and other methods are commonly used for the separation and purification of natural blue pigments and analytical identification. Some natural blue pigments have antioxidant, antitumor, hypolipidemic, hypoglycemic, anti-inflammatory, anti-cancer, immunomodulatory activities, etc. There will be a broad development prospect for the future research of natural blue pigments.

Keywords: Pigments, Natural blue pigment, Microbial blue pigment, Extraction and isolation techniques, Biological activity.

1. Introduction

Colorants, also known as colorants, are substances that can color the mordant, and are widely used in the food, pharmaceutical, cosmetic, and textile industries.[1] . In the past, synthetic pigments were mainly used to produce the required products, and they dominated the market due to their low price, excellent performance and ease of use. With the improvement of science and technology, people are gradually aware of the potential hazards of synthetic colors to the environment and human health.[2] Synthetic pigments not only produce toxic waste, but also have a negative impact on human health. Synthetic colors not only produce toxic waste, but also cause skin allergies, neurological disorders in children and even cancer.[3] In recent years, synthetic colors have become increasingly popular. In recent years, the use of synthetic colors is gradually declining and the demand for natural colors is increasing day by day.[4] Natural colours are derived from substances found in nature. Natural colors are a new alternative to synthetic colors, prepared from substances found in nature.[5] They are safe, reliable and environmentally friendly. They are popular among consumers because they are safe, reliable, non-polluting to the environment and have no toxic side effects.[6] Natural coloring is mainly derived from animals, plants and other plants. Natural pigments are mainly derived from animals, plants, minerals and metabolic substances produced by microbial culture, etc.[7] The natural pigments are mainly derived from animals, plants, minerals and metabolites produced by microbial culture.

Japan is the most advanced country in the world in terms of research into the use of natural food coloring, as a representative of the more stringent requirements for product quality and environmental efforts[8] Japan is the most advanced country in the world in terms of research into the use of natural food coloring, followed by the United States, where large amounts of natural red and natural yellow pigments have been identified in past research.[9] . The coloring effect of natural pigments is closer to the original color of the substance, with the improvement of the level of science and technology, China has also conducted a lot of research and exploration of natural food coloring, to a certain extent to expand the range of varieties of pigments available, according to statistics have been up to more than forty typical pigment varieties, common pigments such as curcumin, red currant red, tea yellow pigment, etc.[10] . While the blue pigment as one of the three primary colors has rarely been reported. Blue is a rare color in nature, the development of blue pigment to seize the international first opportunity to become the goal of domestic and foreign research workers[11] In the long run, natural blue pigment is important for food. In the long run, natural blue pigment has a broad development prospect for food processing, medicine and health care, textile and industrial production of colorants, especially for its biological activity.[1] The blue pigment has a broad development prospect for food processing, medicine and health care, textile and industrial coloring agent, especially for its biological activity. China's vast geographical area and abundant resources have great advantages for research and development of new blue pigment sources. However, natural blue pigments are mostly characterized by unstable chemical properties[12] This requires in-depth research on the extraction and purification process, but there is still no denying that natural blue pigments have a broad market prospect in the future development.

2. Natural Blue Pigment

Most of the currently known natural blue pigments are derived from plants and animals, such as the preparation of gardenia blue pigment from gardenia[13] The phycocyanin is extracted from microalgae[14] In addition to this, indigo dyes are also prepared from the leaves of wood blue plants such as Polygonum multiflorum, Chaenomelanum, Malanum, Wu blue and Woad.[15] . Genetic engineering techniques for genetic modification to induce plants to produce blue substances, but due to the high cost and low efficiency of this technology, it has not been released into industrial production[16] . In the past reports, the most widely available edible natural blue pigments that account for the edible natural blue pigment market are algae blue pigment and gardenia blue pigment, with Spirulina being the main raw material for the production of algae blue pigment[7] .

Spirulina is rich in protein, carbohydrates, lipids, carotenoids and algal bile proteins, among other important components.[17,18] .

C-Phycocyanin (C-PC), a natural blue pigment, is known as the most abundant photosynthetic pigment in Spirulina.[17-19] It is often used in processed food, pharmaceutical and cosmetic industries.[17] . Gardenia Blue is a natural colorant widely used in confectionery, baked goods, frozen desserts, beverages (wine) and jams.[20] . It is produced by adding β-glucosidase to a mixture of cyclic enzyme glycosides extracted from gardenia fruit to produce kynepin[21] Although kynepin itself has no color, it can react with protein hydrolysis products to produce a blue substance, providing a new natural dye for the textile, cosmetic, and food industries.[9,22] . In addition to this, the preparation of natural blue pigments using metabolites produced by microbial cultures is now a major research target[23-25] .

3. Microbial Natural Blue Pigment

In recent years, several pigments of microbial origin have been studied, such as the production of yellow and red pigments in the liquid fermentation of Aspergillus oryzae[26] , extraction of melanin from Aspergillus fumigatus in the environment[27] , the production of yellow carotenoids using a complex fermentation strategy from liquid to solid fermentation with the filamentous fungus Aspergillus pulsatilla, using dilute wine lees and waste bread as substrates, while achieving the conversion of waste to high-quality feed containing pigments[22] , and carotenoids from rusty fungus[28,29] .

However, most of these microbial pigments are red or yellow in color. To date, only a few blue pigments from microorganisms have been reported, and the availability of natural blue colorants for industrial use remains limited. The extraction of most natural blue pigments currently available in the market involves the involvement of microorganisms. Algal microbial sources of natural blue pigments have been found to be mainly eukaryotic algae, red algae and cryptophytes[17,30] , and the cyanobacterium Arthrospira (Spirulina obtusa) [19] Cyanobacteria are responsible for the production of phycocyanin, one of the most important blue compounds approved for use in food and cosmetics.[31] .

In addition, past reports include some studies on other bacteria such as Streptomyces, Pseudomonas, Pseudoalteromonas, purple non-sulfuric bacteria, Durylobacter, and sprouting stunt molds that can produce different blue pigments using microbial substrates.[7] .

In the last few years, research on the production of natural blue pigments by bacteria has been slow and few new strains have been discovered, e.g. the latest research includes the fermentation metabolite of Streptomyces by ethyl acetate extraction, purification by preparative thin layer chromatography and high performance liquid chromatography using mass spectrometry and nuclear magnetic resonance to identify the compound as a new blue pigment from the genus Streptomyces[23]

The strain identified as Pseudomonas aeruginosa was isolated and purified in culture and it was found that the strain produced a water-soluble blue pigment consisting of four main components, the pigment showed high water solubility and very low toxicity, but further studies are needed to improve the level of pigment production and its suitability as an industrial dye[24] .

The production of the blue pigment indigo has been achieved in the entomopathogenic bacterium Luminobacterium luminescens through promoter exchange and heterologous expression of the biosynthetic gene indC in E. coli, and the activation of "silent" gene clusters through genetic engineering techniques, but the research is still at the laboratory stage and there is still a long way to go before industrial production using these microorganisms can be achieved. There is still a long way to go[32,33] The research is still at the laboratory stage and there is still a long way to go before these microorganisms can be used for industrial production. There is also some research on the extraction of natural blue pigments from fungi, which can be grown in a short period of time and can be easily cultured to produce high density cultures, many of which are aromatic polyketides produced during secondary metabolism, and could be an additional source of natural blue pigment production.[18,34] .

For example, red polyketide compounds derived from the secondary metabolism of the fungus Fusarium spp., which obtain a red product during fermentation and turn blue after heat treatment (Brazilian patent BR 10 2013 015305-2 A2). [34] .

Two different fungi were co-cultured to improve pigment production by changing medium conditions and taking different light irradiation, however, despite studies on this aspect, research on improving pigment production and optimizing production protocols for other pigment-producing fungal species has remained superficial, so it has become necessary to study the factors that induce the pigment-producing capacity of fungi[35] .

Although research on the use of microorganisms to extract natural blue pigments still ushers in many problems such as few sources of strains, culture protocols and isolation and purification methods have not yet reached the ideal requirements, but compared with other commonly used methods, the pigments produced by microbial fermentation processes still have many advantages, such as high production efficiency, large culture scale, not affected by climatic and environmental factors, which all make microorganisms as a natural blue pigment This makes microorganisms as a source of natural blue pigment provide a broader development prospect[36] .

For microbial production of edible natural pigments, solubility, color, stability and safety are key factors for meeting the conditions for industrial application. [37] . This requires that the screening and cultivation of the strain and subsequent research on the extraction and isolation and purification of the pigments should take into account whether the content of this research can be applied to industrial production and future processing and utilization.

In the study of natural blue pigment extraction using microorganisms, the research includes the screening and identification of strains, optimization of fermentation media, determination of physical and chemical properties of blue pigment and detection of some toxicological properties, such as the effect of temperature, light, PH, metal ions, oxygen radical scavenging, complexes and additives on the stability of the pigment, as well as antibacterial activity and cytotoxicity.[7,23,25] . However, the number of studies on blue bacterial pigments is limited, mainly because only very few bacteria are able to produce blue pigments[23,36] . Therefore, it is important to screen for new blue pigment producing strains in future research efforts. More in-depth studies on this aspect are needed for a wider application of natural blue pigments in industrial production.

4. Extraction of Natural Blue Pigment

Natural blue pigments are susceptible to structural degradation and changes when subjected to external factors such as light, temperature and pH[9] , so it becomes essential to study the extraction conditions of the pigments. It was found that the methods of extraction of biological compounds from microalgae include supercritical fluid extraction[38] microwave-assisted extraction[39] extraction, pressurized liquid extraction[40] ultrasonic assisted extraction[41] and pulsed electric field treatment.[42] The extraction process was carried out to maintain the bright blue color and antioxidant properties. During extraction, their stability can be improved by adding high concentrations of sugars and salts, such as glucose and sodium chloride, in order to maintain their bright blue color and antioxidant properties.[43] . It has also been shown that light emitting diode lamps and the use of lamp color changes can significantly improve the production of blue pigments[44] . Almost all of these methods used to purify phycocyanins include steps such as precipitation, centrifugation, dialysis, ion exchange chromatography, and gel filtration chromatography, which are both difficult and expensive for application to large-scale production[14,45,46] . However, the current extraction protocols still do not allow the evaluation of all aspects of the biological activity of the extracted blue pigments, and further studies on the chemistry of algal bile proteins with stabilizers and food matrices are needed. In addition, the bioavailability and biological potential of algal bile proteins and their mechanisms of action need to be further investigated.[47] . The extraction process of gardenia blue pigment that is widely used now includes two kinds, one is the one-step process of fermenting gardenia blue pigment by inoculating the enzyme-producing strain into the aqueous extract of gardenia fruit powder, and the other is the two-step process of concentrating the aqueous extract of gardenia fruit powder, followed by the enzymatic reaction to produce gardenia blue pigment[7] . Due to the problem of easy degradation of natural blue pigment, the problem of light resistance, heat resistance, acid and alkali resistance of gardenia blue pigment and stability to different metal ions in the extraction process still need to be studied in depth[48] Recently, a new safety assessment on the consumption of gardenia blue pigment was made again, and the results showed that gardenia blue pigment and its precursor kynepin do not cause damage to human genes[13] However, there are few studies on the modification of natural blue pigment in the current research. The research in recent years has mostly focused on the production of blue pigment using microbial fermentation, and its extraction scheme has been optimized along with the research, and several extraction methods that are representative in the literature are summarized in Table 1.

source (of information etc)	Type of pigment	solvency	extraction and separation	purification and identification	Extraction effect	bibliography
Pseudomonas aeruginosa	extracellular pigment	Soluble in water, partially soluble in methanol, insoluble in other organic solvents	high speed centrifuge Vacuum drying rotary evaporation Vacuum concentration Reversed-phase solid-phase extraction	High performance liquid chromatography UV-Vis absorption spectrum infrared absorption spectrum nuclear magnetic resonance hydrogen spectrum	Extraction rate 2.5 gL-1	[24]
streptomycete (genus Streptomyces)	extracellular pigment	Soluble in alkaline aqueous solutions and some organic solvents	high speed centrifuge Vacuum drying Semi-preparative high performance liquid chromatography	High performance liquid chromatography nuclear magnetic resonance spectrum mass spectrometry	Extraction rate 3gL-1	[25]
	extracellular pigment	Soluble in organic solvents, insoluble in water	high speed centrifuge decompression evaporation Ethyl acetate extraction Thin layer chromatography (TLC) Silica gel column chromatography	High performance liquid chromatography mass spectrometry nuclear magnetic resonance spectrum	Extraction rate 4gL-1	[23]

5. Biological Activity of Natural Blue Pigments

Some natural blue pigments such as phycocyanin (C-PC), in addition to their function as colorants, have antioxidant[49] , anti-tumor[50] , hypolipidemic[51] antioxidant, antitumor, hypolipidemic, anti-inflammatory, anticancer, immunomodulatory[52] The activity of phycocyanin is also very important. Experiments have shown that phycocyanin is a water-soluble fluorescent pigment, consisting of two different polypeptide subunits[53] It can activate cellular antioxidant enzymes, inhibit lipid peroxidation[54] It can activate cellular antioxidant enzymes, inhibit lipid peroxidation and DNA damage, scavenge free radicals (hydroxyl and peroxyl radicals), and increase superoxide dismutase and catalase activities.[44,55] The results of the study confirmed that the treatment was able to activate cellular antioxidant enzymes and inhibit lipid peroxidation and DNA damage. In vivo animal models have confirmed that phycocyanin does not cause acute and chronic toxicity.[31] . Therefore, phycocyanin is also a natural dietary antioxidant that can be used as a supplement and food additive to prevent some chronic diseases involving reactive oxygen species[50] . In addition, it plays a key role in the treatment of many diseases such as diabetes, obesity, arthritis, anemia, cardiovascular disease, allergies, tumors and cancer[56] . The product of hydrolysis of gardenia glycosides, kynepin, can be used as a precursor of natural blue pigment[13] It is also an excellent natural biological cross-linking agent, and biomaterials made from it can help in the treatment of vascular diseases, diabetes, liver dysfunction, etc.[57,58] It is widely used in the biomedical field. In addition, kynepin and its related cyclic enol ether glycosides are a promising drug option that has been found to have strong antioxidant, anti-inflammatory and neuroprotective properties and can be used as a direct drug for the prevention and treatment of liver disease[59,60] . Bicuculline, produced by Fusarium acnes, can turn blue after heat treatment[61] As another source of natural blue pigment, it has been reported in the past to have anticancer, antitumor, antibacterial, and antibacterial properties.[62,63] , antibacterial[64] The important biological activities such as anti-cancer, anti-tumor and anti-bacterial have expanded its use in the pharmaceutical and medical fields. Streptomyces azure is an actinomycete capable of synthesizing blue pigments, and one product, actinomycin, is an antibiotic produced by Streptomyces azure that inhibits most gram-positive bacteria, such as Staphylococcus aureus, at relatively high concentrations and has antioxidant effects under acidic conditions[23,25] . Based on the above studies, the development of new natural blue pigments not only can be applied to the food processing industry as coloring agents, but also have potential medicinal value, therefore, natural blue pigments have a broad development prospect and research value.

6. Conclusion and Future Perspectives

Although natural edible blue pigments occupy many advantages in industrial production and in food and health care, there are still many limitations to the large-scale production of natural edible natural blue pigments in the current state of development. First, there are limited sources of substances from which natural blue pigments can be produced. Apart from extraction from animal and plant tissues, there are few microorganisms that can be used to produce blue pigment, and the scarcity of raw materials can easily cause problems such as the high price of natural blue pigment. And in today's competitive market, people will not consider natural pigments as an additive option to be placed in the market for production. Therefore, finding new sources of strains that can produce natural blue pigments has become a primary goal. Secondly, the instability of natural blue pigments brings a lot of inconvenience to the process of extraction and purification, which increases the energy consumption of the extraction process, while the low extraction rate and the complexity of the purification process make the production cycle long and labor-intensive. Therefore, based on the existing raw materials, improving the extraction scheme, increasing the production capacity and perfecting the purification process are also essential elements for the production of natural blue pigment. Thirdly, the safety of natural blue pigment cannot be ignored when it is applied to food processing as well as medical and health care industries. Especially for the food industry, the solubility and safety of natural blue pigment directly affect the quality of the product, which is a key issue concerning personal health. Therefore, toxicological, carcinogenic and other safety studies should be strictly conducted before use to determine safe doses and exclude interactions between components to ensure that the product does not pose a health hazard. Despite all the limitations of the research on edible natural blue pigments, the current situation shows that the use of natural additives will be a necessary issue in the future in the food and medical field, and consumers will tend to prefer natural additives more. In view of this, developing new sources of strains, improving the production process and increasing the feasibility analysis and testing of natural blue pigments will be a major development goal for natural additives in the future, and deepening the research on scarce natural blue pigments will have far-reaching research significance and broad development prospects.

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