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Biocolorants from Plant Sources: A Novel Approach to Enhance the Aesthetics and Health Profile of Foods

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Abstract: Color plays an essential role in the appeal and overall quality of food items. Throughout the processing of food, a significant amount of color is lost, prompting the addition of synthetic or natural colors to make the food visually enticing to consumers. Bio colorings, derived from renewable sources and often originating from plants, offer a sustainable alternative. Plant-based colors, recognized as a natural and ideal source of color, have the potential to replace many synthetic counterparts. Beyond their application in food coloring, bio colorings also serve as antimicrobials and antioxidants, contributing to the prevention of various conditions and diseases in humans. The attention garnered by bio colorings is due to their eco-friendly nature, safety, and versatile applications in various industries, particularly in the vibrant field of coloring. This paper explores diverse sources of colorings, their applications in fabrics and food, and the inherent advantages they offer in promoting sustainability. The development of cost-effective and practical technologies for the extraction and utilization of natural food colorants is a significant challenge and a pressing need in the current landscape. The paper addresses the challenges and future prospects of colorings, emphasizing their role in mitigating the environmental impact associated with synthetic colorings.

Keywords: Biocolorants, natural pigments, sustainable colorants, extraction techniques, eco-friendly dyes, industrial applications.

1. Introduction

In recent years, there has been a growing awareness and demand for natural and sustainable alternatives in various industries, including the food sector. One significant area of interest is the utilization of bio colorants derived from plant sources as a novel approach to enhance the aesthetics and health profile of foods. The shift towards plant-based ingredients in the food industry aligns with consumer preferences for clean labels, environmental sustainability, and improved nutritional content.Biocolorants refer to pigments obtained from botanical sources, such as fruits, vegetables, and herbs. These natural pigments not only contribute vibrant hues to food products but also offer potential health benefits due to their inherent bioactive compounds. Unlike synthetic colorants, which may raise concerns related to safety and environmental impact, bio colorants from plant sources are generally perceived as safer and more environmentally friendly.Bio colorants impart a spectrum of colors to food products, ranging from deep reds and greens to vibrant yellows and purples. This natural diversity allows food manufacturers to create visually appealing products without the need for artificial additives. Consumers often associate natural colors with freshness and authenticity, reinforcing the appeal of bio colorants in the market.Plant-based colorants often come with additional health benefits attributed to the bioactive compounds present in the source plants. For instance, anthocyanins found in berries not only contribute to the red and purple hues but also possess antioxidant properties that may contribute to health and wellness. By incorporating bio colorants, food products can potentially offer both visual appeal and nutritional value.The clean label trend, emphasizing transparency and simplicity in ingredient lists, has prompted food manufacturers to seek natural alternatives to synthetic additives. Bio colorants align with this movement, providing a clean label option that resonates with consumers looking for wholesome and minimally processed foods.Plant-based colorants contribute to environmental sustainability by reducing reliance on petroleum-based synthetic dyes. Additionally, sourcing colorants from agricultural by-products or waste materials can promote a circular economy and minimize environmental impact.The regulatory landscape governing food colorants is evolving, with increased scrutiny on synthetic additives. Bio colorants, being derived from natural sources, may enjoy favourable regulatory considerations, further supporting their adoption in the food industry.The use of synthetic colorings in diligence similar as fabrics, food, and cosmetics has raised enterprises about their adverse environmental and health goods. Color has a significant biocolorings, which encompass natural colors deduced from shops, microorganisms, insects, and other natural sources. This section introduces the significance of colorings in the environment of growing sustainability enterprises.The use of colorings has gained elevation due to theireco-friendly nature andeventuality to replace synthetic colorings.Currently, a large number of consumers have a strong preference for the "functional foods" for the enhancement of their eating diet and conservation of their health(Shashirekha etal., 2015). Consequently, fruits and vegetables are important corridor of the healthy diet with the capacity of precluding several conditions(Shashirekha etal., 2015). Biocolorants, also known as natural colorings or bio-based colorings, are colorcomposites deduced from natural sources, similar as shops, microorganisms or insects. These natural colorings serve several purposes in colorful diligence and operations,food and beverage industry, textile industry, cosmetics and personal care products, pharmaceutical, art and craft, agriculture and horticulture, environmental operations, research and education, sustainability and eco-friendliness, cultural and traditional uses.

2.0 Classification of Colors

2.1 Natural Identical Colors

Nature identical colors have been developed to match their counterpart in nature. The most colors that are synthesized are carotenoids conforming of conjugated hydrocarbons, and as similar they prone to oxidative attack and posterior loss of color. carrying colors from natural sources can be much more expensive and their quality can vary. To overcome this, druggists have set up ways to make identical colors in the laboratory. This improves their chastity and may also bring lower. Nature identical colors are exactly the same motes set up in natural sources but they're made synthetically. E.g., Flavonoids, set up in numerous flowers, fruits and vegetables, indigoid, set up in beetroot.Carotenoids, set up in carrots, tomatoes, oranges and utmost shops. Carrots contain an orange patch called beta carotene which is part of this group.
2. Synthetic colors these are synthetic colorings, which are organic chemical substances. These days they're manufactured either from coal navigator derivations or petrochemicals,e.g., brilliant blue, erythrosine.

Table 1 -Different types of colors and applications

Type	Name	Application	References
Natural identical colors	E100	Curcumin (Yellow)	Sigurdson, Gregory T., Peipei Tang, and M. Mónica Giusti. "Natural colorants: Food colorants from natural sources." Annual review of food science and technology 8 (2017): 261-280.
	E101	Riboflavin (Vitamin B2) (Yellow)	
	E120	Cochineal (carmine) (Red)	
	E163	Anthocyanins (Red)	
	E170	Calcium carbonate(white)	
	E172	Iron oxides and Hydroxides (Black,Red,Yellow)	
Bio colorants from plant sources	Annato	Brightening of cheese	Rymbai, Heiplanmi, Ram Roshan Sharma, and Manish Srivastav. "Bio-colorants and its implications in health and food industry–a review." International Journal of
	Turmeric	Coloring pickles, pudding, confectionary	
	Carthamus	Beverages and dressing deserts	
	Xanthophyll	Great stability towards pH, light and heat	
	Beta-carotene	Pro vitamin A activity	
	Paprika oleoresin	Use in soups, snacks, sausages	
	Red-beet	Yogurt, ice-cream, confectionary	

	Chlorophyll	Liquors and jellies	Pharmacological Research 3.4 (2011): 2228-2244.
Bio colorants from insect sources	Cochineal/carmine obtained from Dactylopius coccus	Orange	Gupta, Charu, et al. "Microbes as potential source of biocolors." Pharmacolo gyonline 2 (2011): 1309-1318.
Bio colorants from microbial sources	Staphylococcus aureus	Zeaxanthin	
	Xanthomonas oryzae	Xanthomonadin	
	Phaffia rhodozyma	Astaxanthin	
	Serratia rubidaea	Prodigiosin like pigment	
	Dunaliella salina	ß carotene	
	Monascus roseus, Monascorubramin,	Monascus spp. Canthaxanthin Rubropunctatin Ankaflavin	

2.2 Bio colorants from plant sources

Plants contain pigments that give them their natural vibrant colors. Common pigments found in vegetables and fruits include red-yellow betalains, red-purple anthocyanins, green chlorophylls, and yellow orange carotenoids. In addition to providing color, these plant pigments may offer health benefits and help prevent certain diseases. Agricultural waste products are an important source of natural pigments that are increasingly viewed as sustainable resources. Utilizing these residues for pigment extraction could help reduce environmental issues associated with their disposal. Fruit and vegetable peels and seeds, which are typically discarded during processing, can still contain significant amounts of pigments, often in higher concentrations than the edible portions. (Sharma, Usmani, Gupta, & Bhat, 2021).

2.3 Bio colorants from insect sources

Cochineal/Carmine extracts are derived from the shield of the insect Dactylopiuscoccus, which is cultivated in South America. The coloring pigment, carminic acid, has been used for coloring clothes and food since ancient times. Cochineal is primarily used in low pH products like confectionery, beverages, and liquors, providing an orange hue. Carmine is a lake pigment produced from carminic acid and is utilized in cosmetics, confectionery, meat products, and various other foods. Both cochineal and carmine exhibit excellent light and heat stability, making them ideal for food products exposed to direct light.

2.4 Bio colorants from microbial sources

Monascus colors are derived from the fungus Monascus purpureus, traditionally grown on steamed rice through solid-state fermentation. They are commonly used as natural coloring agents in traditional and oriental foods. According to Poorniammal et al. (2021), Monascus colors can be used to color banana sauce and tonica at concentrations of 1.5% and 1%, respectively.Phycocyanin, another natural colorant, imparts a blue hue and is typically extracted from Spirulina sp. It is known for its high stability in pH ranges of 5-7 and is often used as a colorant in frozen desserts, sherbets, and confections.Beta-carotene is sourced from Phycomyces blakesleeanus. While the wild type of Phycomyces blakesleeanus grown under standard conditions has a modest carotene content of about 0.05 mg per gram of dry mass, certain mutants can accumulate up to 10 mg per gram of dry mass.Zeaxanthin, extracted from Flavobacterium sp., is a yellowish colorant known for its use in flesh feeds to enhance the skin color of animals or to intensify the color of their egg yolks. It is also used as a colorant in cosmetics and the food industry.Astaxanthin, obtained from Phaffia rhodozyma, is widely distributed in nature and is responsible for the distinctive orange-red color in crustaceans and salmonids. It is highly valued for its consumer appeal in the market.Zeaxanthin from Dunaliella salina, a single-celled, saltwater micro-alga, accumulates large amounts of carotenoids under suitable growth conditions. Natural mixed carotenoids found in Dunaliella salina, including Beta Carotene, Alpha Carotene, and Xanthophylls like Zeaxanthin, Cryptoxanthin, and Lutein, are renowned for their antioxidant properties. Different types of microbial sources of obtaining bio colors as discussed in table no.2.

Table -2 Biocolorants from microbial sources

Dyes	**Utilization**	**Pigment**	**Microbes**	**Biological activities**
Algae				
Red-orange	Cheese, pastry, ice-cream.	β-carotene	Dunaliella salina	Anticancer, antioxidants.
Red	Feed of poultry	Canthaxanthin	Hematococcus	Antioxidant
Blue	Neutraceuticals, biotechnology, cosmetic & food industries	Phycocyanin	Arthrospira sp.	Antioxidants, antitumors, and immunoregulatory.

Bacteria				
Purple	Medicinal, cosmetic and food industry	Violacein	Janthinobacterium lividum, Chromobacterium violaceum	Antioxidant
Yellow	Energy drinks, infant food, sauces, cereal products, & Food products	Riboflavin	Ashbya gossip	Protection of cardiovascular, antioxidant & anticancer
Orange, Pink	Poultry feed	Canthaxanthin	Monascus spp.	Antioxidant and anticancer
Fungi				
Yellow-Orange	Pastry, cream, cheese & ice cream	β-carotene	Mucor circinelloides, Neurospora crassa, and Phycomyces blakesleeanus	Anticancer, antioxidant
Pink-Red	Cosmetic, fish, and animal food products	Astaxanthin	Agrobacterium aurantiacum, Paracoccus, carotinifaciens, Xanthophyllomyces dendrorhous	Anti-inflammatory, anticancer& antioxidants
Yeast				
Black	Cosmetic, medical & food industries	Melanin	Saccharomyces, Neoformans	Antibiofilm, antioxidant & anti-microbial.
Pink-Red	Fish & animal products, foods & cosmetic industry	Astaxanthin	Xanthophyllomyces diandrous, Phaffia rhodozyma	Anti-inflammatory, photo protectant, anticancer
Actinomycetes				
Red	Astaxanthin, carbonated drink, milk, yogurt, cereal, ice cream cones	Prodigiosin	Streptoverticillium rubrireticuli	Antioxidant and anticancer.

Need of bio colorants-The need for natural bio colorants has arisen due to the health risks associated with mineral-derived colors like lead chromate and barium sulfate, which can pose serious health hazards and environmental risks. Over the past few decades, synthetic colorants have faced heavy criticism, leading consumers to prefer natural alternatives. Environmental activists have campaigned against synthetic colorants, highlighting their potential health and environmental impacts. This has led to a significant reduction in the number of permitted artificial colors, with consumers showing a greater interest in natural colorings.

The shift towards natural colorants can be attributed to various factors, including concerns about health, nutrition, pharmaceutical standards, fashion trends, and environmental awareness. Natural colors are also preferred for their vibrant appearance and perceived nutritional benefits. As a result, natural colorings have become the preferred choice over synthetic colorants.The use of colorings in food serves several purposes, including maintaining the original appearance of food after processing and during storage, ensuring color consistency to avoid seasonal variations, preserving the natural color of food to maintain its quality, protecting flavors and light-sensitive vitamins, and enhancing the appeal of food as an appetizing item(Ghosh et al., 2021).

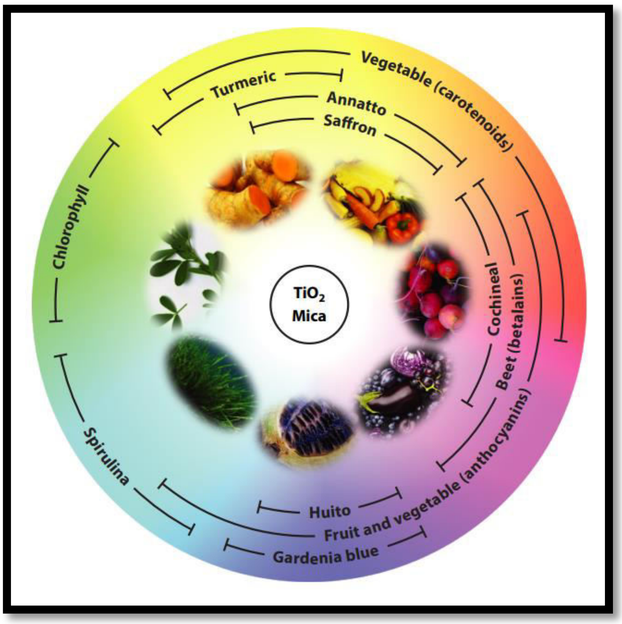


Fig. 1 Pie chart showing different types of colors

2.5 Structure and Composition of betalain

IUPAC (International Union of Pure and Applied Chemistry) name of betalains is (2S)-1-{2-[(2S)-2,6-dicarboxy-2,3-dihydropyridin-4(1H)-ylidene] ethylidene}-5-(β-d-glucopyranosyloxy) -6-hydroxy-2,3-dihydro-1H-indol-1-ium-2-carboxylate.Betalains are nitrogen-containing pigments that can be classified into two main groups: betacyanins and betaxanthins. Each group consists of specific compounds, and their chemical structures contribute to the distinct colors they impart such asthe purple pigments are betacyanins; betaxanthins are orange to yellow colored (Bocker and Silva 2021). Both pigments are characterized by one moiety derived from betalamic acid, a key component in the structure of both betacyanins and betaxanthins. Betalain molecules differ from each other by the part bound to the betalamic acid residue. The chemical structure of betacyanin includes a central chromophore known as betalamic acid, which is responsible for the color, and a sugar moiety, often glucose. In betacyanins of higher plants this moiety is provided by cyclo-DOPA. Its O-glycosidation and acylation results in the formation of a large variety of purple pigments and make it heat stable.

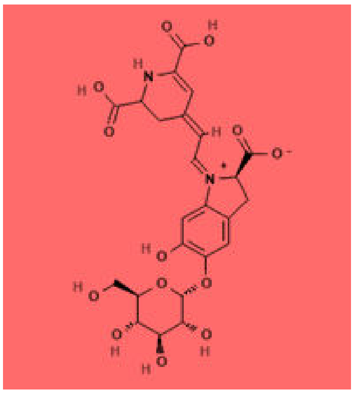


Fig.2Betalains

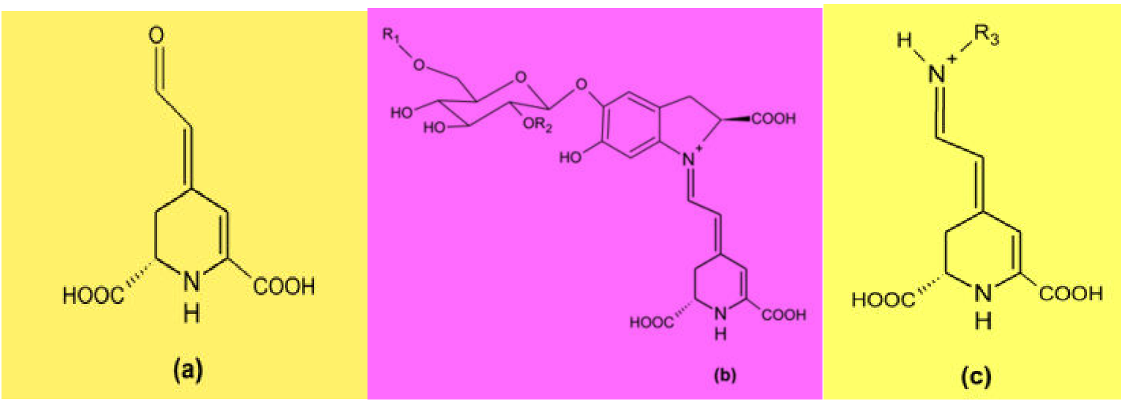


Fig. 3 General structureof (a) betalamic acid (b) Betacyanin's and (c) Betaxanthins. Betanin: R_1 =R_2=H. R_3= amine or amino acid group

3.0 Betalains as bio-colorant

Betalains are a group of compounds that have great potential for enriching and supplementing foods due to their pigmentation, antioxidant properties, antimicrobial effects, and other bioactivities associated with potential health benefits for humans. Adding betalains to products can increase their polyphenol content, thereby boosting antioxidant properties. Betalains are highly hydrophilic due to their hydroxyl groups, which allow for charge polarization and hydrogen bond formation.Betalains have gained attention for their antiviral and antibacterial properties. They are produced by the conjugation between betalamic acid and a primary or secondary amine. Betalains are divided into two main classes: betacyanins (red/violet) and betaxanthins (orange-yellow). Their stability is influenced by factors such as sugar, light, oxygen, water activity, pH, and temperature. Betalains are not heat-resistant, so foods containing them are kept in low-light, low-oxygen, and low-humidity environments.These colorants are found in fruits, flowers, stems, roots, bracts, and certain genera of higher fungi belonging to the Caryophyllales order. Food sources of betalains include red beetroot, dragon fruit, cactus fruit, amaranth, and radish.(Brudzyn´ska et al. 2021).

The most common betacyanin is betanidin-5-b-glycoside(betanin), the main coloring beetroot.(Stintzing & Carle, 2004). Betacyanin's parade two immersion maxes – one in the UV region(270 – 280 nm) due to cyclo- Dopa and the alternate unnoticeable range(535–540 nm, depending on the detergent). Red and grandiloquent colors affect from different negotiation patterns of betacyanin's. Glycosylation betanidin generally comes along with hypsochrome shift of about 6 nm, while the other sugar group attached to the first supposedly didn't affect important color(Cai etal., 1998; Stintzing & Carle, 2004). Acylation with hydroxycinnamic acid produces the third outside(300 – 330 nm), while aliphatic acyl group don't change the diapason(Stintzing & Carle, 2004).Structural variations in betaxanthins beget hypsoor bathochromic shifts. Amine conjugates parade a lower immersion outside than their separate amino acidic counterparts(Stintzing etal., 2002b). Betalains have colorful uses in food. Goodies, aftersdry composites, dairy products, meat producing product. Pure color attention needed. The asked coloris attained in fairly small amounts(infrequently further than 50 mg). kg, calculated as betanin(Delgado- Vargas) etal., 2000). According to the codex Commission(2004), the use ofbetalains is limited only by good product training. Food coloring agents are known as" Beet red" uprooted from beets is capitalized as a food coloring agent in the European Union and the United States(Kastelar etal., 2006). Beet coloring agents are commercially available as concentrated fruit juice(produced by snap drying or spray drying), containing0.3 to 1 color(Serzal etal., 2014; Serzal and Nunez, 2019).

3.2Synthesis and stability of betalains

Betalains are having water soluble nitrogen- containing different types of colors. It's set up in the vacuole of factory cells (Sadowska- Bartosz & Bartosz, 2021), which are farther divided into two structural groups. Betacyanin (red- purple) fig. (a) and Betaxanthin (orange- unheroic) fig. (b) (Miguel, 2018). Beta thalamic acid, all core structures betalins suffer two types of condensation responses. Depending on the betacyanin structure, glycosylation and acylation of the performing 5- O- or 6- O- glucosides lead to different betacyanin structures (Belhadj Slimen etal., 2017). Betacyanin's are classified into four structural types. Betanin, Amaranthine, Gumfernin, Bougainville (Polturak &Aharoni, 2018) Depending on the attachment bond with the oxygen snippet in the o- position of the glucosyl group for the cyclo- DOPA half (Belhadj Slimen etal., 2017).

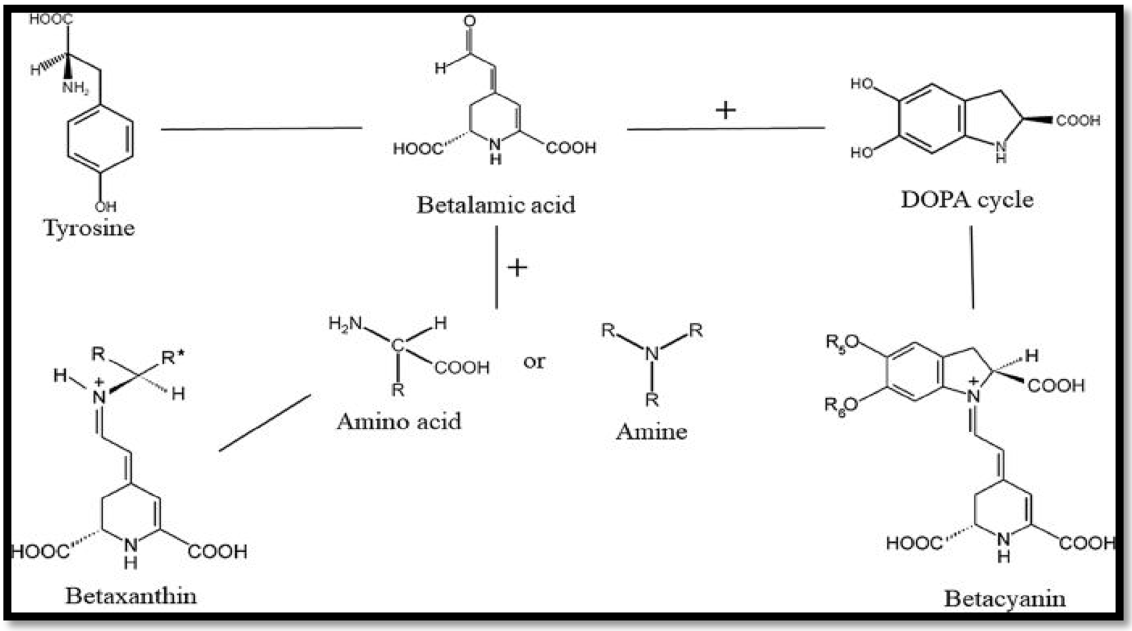


Fig.4-Synthesis of betalains

On the other hand, betaxanthin can be divided into two types. Structural group conjugated group deduced from amino acids and conjugated groups deduced from amines as amino acids. The amine side chain replaces the cyclo- DOPA (3,4-Dihydroxyphenylalanine) unit of the betaxanthin patch (Chang etal., 2015; Miguel, 2018). The difference in chemical structure between betacyanin and betaxanthin has a different maximum immersion thus, color variation occurs. Betacyanin's show two immersion maxes. One is in the UV range from 270 ° to 270 °. 280 nm is due to cyclodopa unit and another visible range from 535 to 540 nm (depending on terrain) (Halal Azerdo, 2009) is the maximum immersion value Betaxanthin varies in the range of 460- 480 nm (Belhadj Sulimen etal., 2017), and structures attached to amine groups(Azerdo, 2009). Different the forms of betalain colors are still being discovered thanks to technological advances and logical styles (Skalicki etal., 2020).

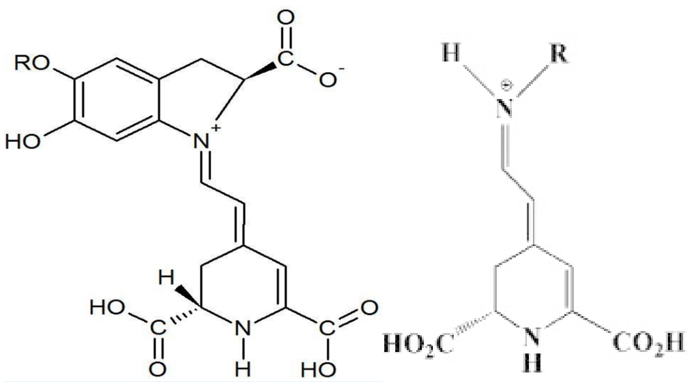


Fig. 5 (a) (b)

In utmost food color operations, an estimated volume of lower than 50 mg betanin/ kg could produce the asked color (Delgado- Vargas etal., 2000). The exercises of betalains have been greatly reduced owing to its poor stability, which poses a significant problem when contending with synthetic colorings (Ozela, 2004). A list of factors that reduce enhance betalain stability is presented in Fig. 5a. Manchali, Murthy, Nagaraju, and Neel warne (2013) listed the possible changes that betalains may suffer during declination, similar as breakdown of the aldimine bond, dehydrogenation, deglycosylation, decarboxylation and isomerisation (Fig. 5b). In order to increase its marketable operations, betalain colors need to repel or decelerate down these changes through stabilization ways.

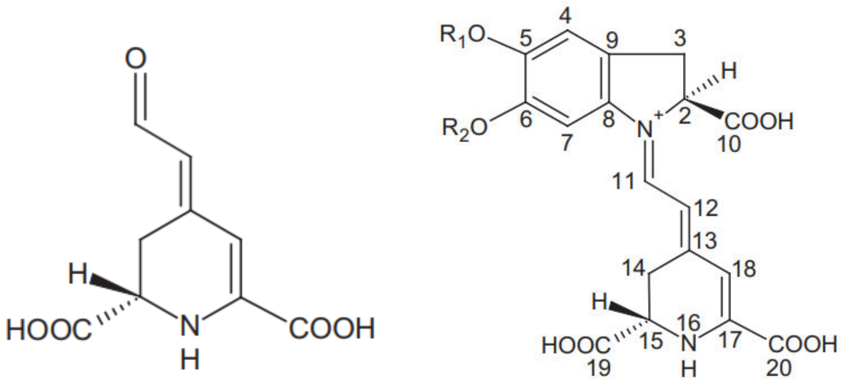


Fig.6(a) Betalamic acid (b)Betacyanin's

3.3Factors affecting chemical stability of betalains

Betalain declination may do by different mechanisms, which were detailed by Herbach etal. (2006b).Several factors, both natural and foreign, affect betalain stability, and need to be considered to ensureoptimum color and color retention in foods containing betalains.

3.3.1pH

Despite changing their charge upon pH change, betalains aren't susceptible to hydrolytic fractionalization as anthocyanins. Betalains is fairly stable over a broad pH range from 3 to 7, which allows their operation to low acidity below food pH 3.5, the immersion maximum shifts there's a shift towards lower wavelengths, and above pH 7 overhead. The stability of betalains is strongly influenced by pH, and their colors can change with variations in acidity or alkalinity. Betalains exist in different forms depending on the pH of the surrounding environment. The pH range over which betalains exhibit stable colors is often referred to as the "pH stability range." Acidic Conditions (Low pH): In acidic conditions, betalains are typically more stable and exhibit their characteristic colors. Betacyanins (red and violet pigments) are stable in acidic to neutral pH conditions, ranging from pH 3 to 7. Betaxanthins (yellow and orange pigments) are also stable in acidic conditions, but they may start to degrade at very low pH values. Neutral to Slightly Alkaline

Conditions: Betacyanins remain stable in neutral to slightly alkaline conditions, with their color intensity gradually decreasing as pH increases beyond 7. However, betaxanthins are more sensitive to alkaline conditions, and their color may shift towards brown or brownish-yellow in pH ranges above 7.

3.3.2 Water activity (aw)

The stability of betalains is significantly influenced by water activity (aw), with aldimine being a crucial factor in determining the susceptibility of colors to bond fragmentation (Saguy et al., 1984; Herbach et al., 2006b). The impact on betalain stability may be attributed to the low mobility or limited oxygen solubility of the reactants (Delgado-Vargas et al., 2000).

Researchers conducted kinetic studies on beet pigments using beet powder stored under air in a desiccator over appropriate saturated salt solutions. They took special care to spread the powder as a thin layer to prevent oxygen from becoming the limiting factor. After equilibration (either adsorption or desorption), they periodically withdrew samples and measured the pigment content. To understand the effect of different moisture contents at specific water activities, they employed two approaches. The first approach involved utilizing the hysteresis phenomenon, comparing samples from the desorption and adsorption sides, which differed only in their water content. The second approach involved combining various levels of beet powder with microcrystalline cellulose or pectin to yield specific ratios. All kinetic studies were conducted at 35°C in complete darkness and in duplicate.

3.3.3Oxygen

Betalains, like many other natural pigments, can undergo reactions with oxygen that lead to degradation and color changes. The reactions with oxygen are typically oxidative processes that result in the loss of the vibrant colors associated with betalains. The primary reactions involving oxygen in betalains include: Oxidative Cleavage of Betalamic Acid: Betalains contain a central chromophore known as betalamic acid. In the presence of oxygen, especially under certain environmental conditions or during processing, oxidative cleavage of betalamic acid can occur. This process leads to the breakdown of the chromophore and results in the loss of the characteristic color of betalains.

3.3.4Temperature

Thermal processing is usually used in development of different processed products. Temperature affects betalains stability and increase in temperature results in betalains degradation as well as degradation of PPO (polyphenol oxidase) enzymes. Temperature had a more significant impact on color indexes (total color difference, chroma, and hue) than UV-light exposure, particularly at 40 °C. s (Saguy et al., 1978; Havlı´kova´ et al., 1983; Garcı´a Barrera et al., 1998). However, thermal degradation is also affected by temperature range, heating extent, oxygen presence, and concentration of pigments (Herbach Stintzing and Carle, 2006).

3.3.5Light

Color degradation occurs in the presence of light. There is an inverse relationship between light intensity in the range of 2200–4400 lux and betalain stability. When exposed to light in the UV and visible range, electrons of the betalain chromophore are excited to a higher energy state, increasing the reactivity or lowering the activation energy of the molecule. However, the effect of light is negligible under anaerobic conditions (Paciulli et al, 2016).

4.0 Betalain extraction

Extraction is a crucial process in natural dyeing and coloring, significantly impacting the concentration of colorants in the final product (Adeel et al., 2018). Scientists have employed various techniques to extract natural pigments responsible for the colors of natural materials. These techniques include boiling and condensation-based extraction (e.g., the Soxhlet method), solvent extraction, ultrasonic-assisted extraction, and microwave-assisted extraction. Newer or advanced technologies are often preferred over traditional methods due to their higher efficiency, increased color yield, and reduced resource consumption in terms of energy, time, and cost.

4.1 Extraction of betalians by Conventional method

4.1.1 Soxhletand Maceration method

Constatin et al. (2021) conducted a study based on spectrophotometric techniques to analyze the impact of conventional solvent extraction on betalains and polyphenolic compounds present in beetroot peels. Various treatments were applied to the beetroot peel samples by varying factors such as ethanol concentration, citric acid concentration, temperature, and time. A Central Composite Design (CCD) was utilized to investigate the effect of these extraction parameters and optimize the extraction of betalains and total polyphenols from beetroot. A quadratic model was proposed and used for all the analyzed parameters. The experimental plan examined the following ranges in coded form: citric acid concentration (0.10–1.5%), ethanol concentration (10–50%), operating temperature (20–60°C), and extraction time (15–50 min). The experimental design revealed betalain content ranging from 0.29 to 1.44 mg/g dry weight (DW), while the polyphenol yield varied from 1.64 to 2.74 mg/g DW. The optimized conditions for maximizing the recovery of betalains and phenols were citric acid concentration of 1.5%, ethanol concentration of 50%, temperature of 52.52°C, and extraction time of 49.9 minutes.

The demerits of uing soxhlet and maceration methods are Time-consuming, High-energy consumption,Potential for thermal degradation, Limited to certain sample sizes, Solvent waste, Incomplete extraction, Risk of sample overheating, Low extraction efficiency, Dependency on solvent, Batch-to-batch variability,

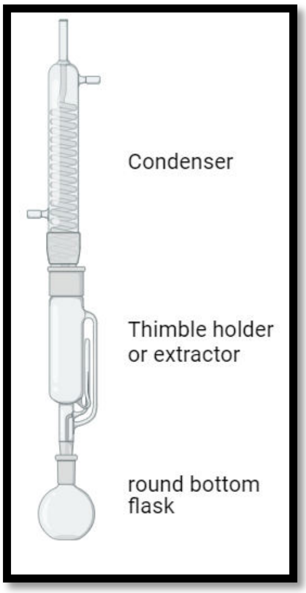


Fig. 7 Soxhlet apparatus

4.2 Extraction of betalians by non-conventional method

4.2.1.Supercritical fluid extraction

Masoud et al. (2023) conducted research to evaluate the effects of microwave power (100-450 W), carbon dioxide flow rate (1-3 mL/min), temperature (30-70°C), and pressure (15-40 MPa) on the extraction efficiency, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and quantities of phenolic compounds extracted from beetroot. The results indicated that temperature, pressure, flow rate, and microwave power significantly influenced the amounts of phenolic compounds, extraction efficiency, and antioxidant properties of the extracted compounds. Increases in these parameters within certain ranges (50-60°C, 20-30 MPa, 300-400 W, and 1-2 mL/min, respectively) improved the response variables, but further increases led to decreases. Optimal betalain extraction from beetroot using supercritical carbon dioxide (scCO2) and microwave pretreatment was achieved at 45°C, 27.5 MPa, 2 mL/min CO2 flow rate, and 300 W microwave power.

Kushwaha et al. (2017) aimed to optimize an eco-friendly method for extracting betalains and phytochemicals from beetroot pomace. They considered different experimental variables: solid-to-liquid ratio (1:15–1:45), temperature (30–70°C), time (2.50–12.50 min), and pH (1.50–5.50) using response surface methodology. Dried, ground beetroot pomace was subjected to extraction of betacyanin, betaxanthin, phenolics, and antioxidants under different combinations of these variables. A prediction model was optimized and validated to determine the optimal conditions, which were found to be a solid-to-liquid ratio of 1:15, temperature of 50.04°C, time of 10 minutes, and pH of 2.50, with a desirability value of 0.889. These conditions enabled the best extraction of betalains and other phytochemicals from beetroot pomace.

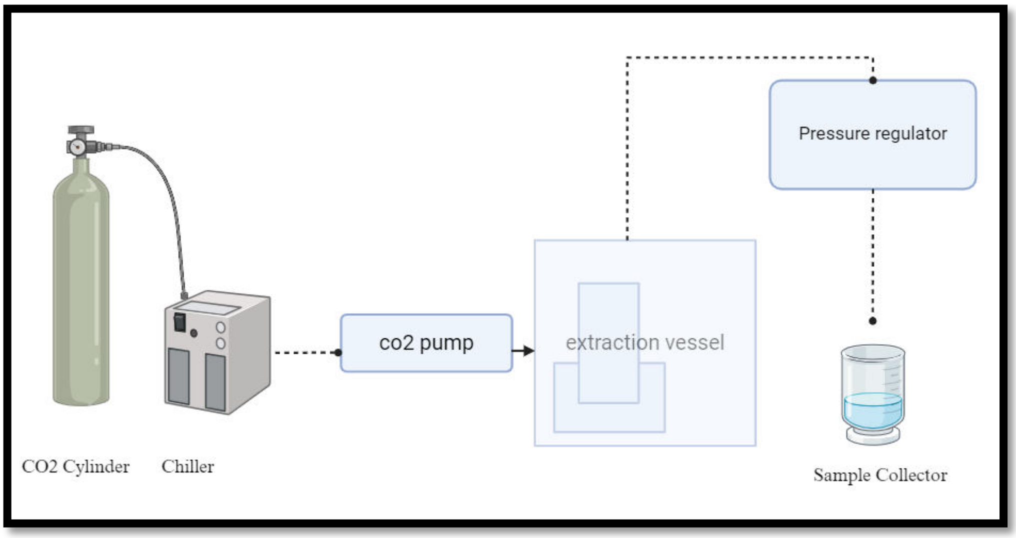


Fig. 8 Super critical fluid extractor

4.2.2 Pulsed electric field

Most likely mechanism of the PEF is the electroporation process. In electro-permeabilization or electroporation, an external electrical force is utilized to increase the mobility of cell membranes (Panja 2018). Pulse electric field (PEF) may assist in the extraction of pigments and coloring agents from various plant-based products (Arshad et al. 2020). Pulsed electric fields have been used to dry fruits and vegetables, extract juice, and inactivate microbes. In this process, an electric field strength of 100–300 V/cm is utilized in batch mode, whereas an electric field strength of 20–80 kV/cm is employed in continuous mode.

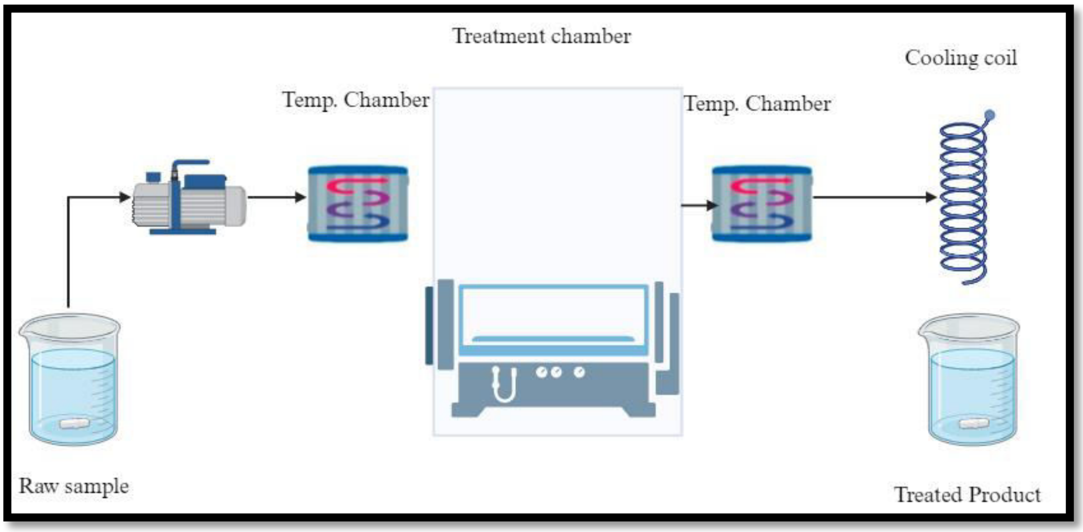


Fig. 9 Pulse Electric Field

Nowacka et al. (2019)studied the influence of pulsed electric field (PEF) pretreatment, at different electric field strengths (4.38 and 6.25 kV/cm), pulse numbers (10–30), and energy inputs (0–12.5 kJ/kg), on the extraction of betalains from beetroot. both findings revealed that PEF pretreatment significantly ($p<0.05$)

affected the extraction efficiencyof bioactive compounds from beetroot. The maximum increase in betalain compounds (betanin by 329%, vulgaxanthin by 244%, compared to the control) in the red beet extract was observed for 20 pulses at 4.38 kV/cm electric field strength.

4.2.3 Ohmic heating for extraction

In ohmic heating-assisted extraction, an electrical current is used to heat the biomaterial. As a result of the sample's electrical resistance, the material gets heated from the core to the outer surface (Sakr and Liu 2014). Ohmic heating (OH) has proved to be highly beneficial in terms of plant digestion. This approach minimizes the number of processing materials undergone while yet obtaining relatively little compositional and color changes (Brochier et al. 2019).

Cabas et al. (2021) Their primary goals of this study were to assess the combined effects of ohmic heating–assisted extraction (OHAE) parameters (voltage gradient and frequency) and green extraction mediums (such as aqueous, aqueous ethanol, and acidified aqueous ethanol) on betalain yield, extraction efficiency, color quality, energy consumption, and exergy efficiency. A comparison was made with conventional extraction (CE) processes. The results showed that betacyanin yield increased with higher voltage gradients during OHAE, with the best extraction achieved in aqueous ethanol.

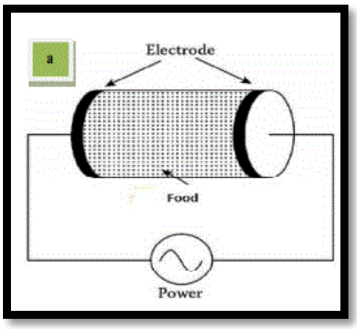


Fig. 10 Ohmic heating device

4.2.4 Ultrasound for extraction

With the advent of ultrasonic extraction technologies, it has become possible to extract components in a matter of minutes with high repeatability. This approach reduces solvent consumption, simplifies manipulation and workup processes, and enhances the purity of the final product. A wide range of compounds, including aromatics, pigments, and various chemical and mineral substances, have been successfully extracted, analyzed, and synthesized using ultrasonic extraction methods (Chemat et al., 2017).

Ultrasonic extraction typically involves the use of sound waves with frequencies exceeding 20 kHz (up to 10 MHz). These mechanical waves can induce vibrations in gases, liquids, and solids. Unlike electromagnetic waves, ultrasound waves undergo compression and expansion cycles as they propagate through a material.Ultrasound waves create cavitation bubbles in the extraction solvent. When these bubbles collapse, they generate localized high temperatures and pressures, leading to the disruption of plant cell walls and facilitating the release of intracellular compounds, including betalains. Ultrasound enhances mass transfer by promoting the movement of solvents into the plant cells and the diffusion of extracted compounds out of the cells. This increased mass transfer accelerates the extraction process. Compared to traditional extraction methods, ultrasound-assisted extraction can significantly reduce the time required to extract betalains. The accelerated extraction process is particularly beneficial for minimizing the degradation of heat-sensitive compounds. Ultrasound helps to break down cell structures more efficiently, leading to higher extraction yields. The increased efficiency is attributed to the disruption of cell membranes and the release of intracellular components into the extraction solvent. Ultrasound parameters, such as frequency and intensity, can be adjusted to achieve selective extraction of specific compounds(Chemat et al. 2017).

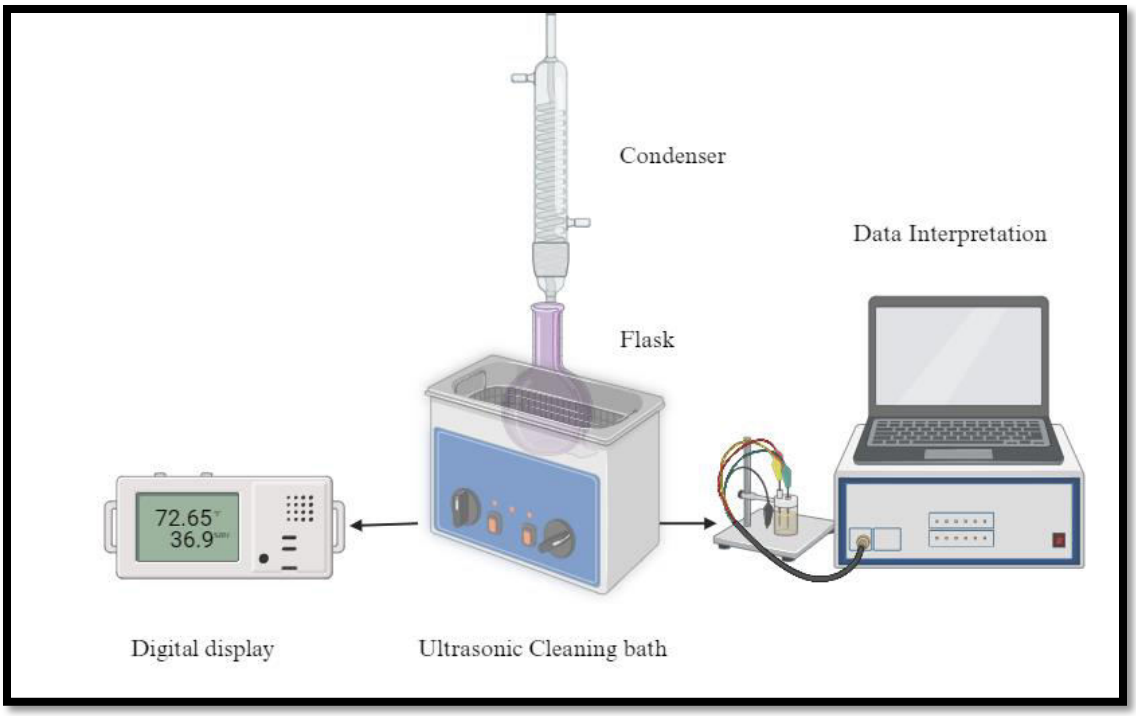


Fig. 11 Ultrasound Assisted Extraction

4.2.5 Enzymes for extracting colors

Enzyme technology has emerged as a novel method for extracting colors. Enzymes are capable of hydrolyzing cell wall components and disrupting structural integrity, forming the basis for enzyme-assisted extraction. The formation of an enzyme-substrate complex, which changes the enzyme's structure to better accommodate the substrate in its active site, can facilitate cell wall hydrolysis. This leads to the dissolution of cell wall constituents as a result of these conformational changes, ultimately breaking down the cell wall attachment

(Manzoor et al., 2021). Enzymes play a crucial role in pigment extraction, enhancing the quality and uniformity of the final product. (Sharma et al. 2014).

Lombardelli et al. (2021) Food waste management is important for the circular economy. This study focused on using unsold red beets from supermarkets as a source for extracting natural betalain colorants, avoiding organic solvents. An enzymatic mix tailored to the beetroot cell wall composition (cellulase 37%, xylanase 35%, pectinase 28%) was used for enzyme-assisted extraction. The extraction protocol was optimized, and the best conditions for maximum betalain yield and desirable color attributes were: 25 U/g total enzyme dose, temperature 25°C, and 240 minutes processing time. The aim was to develop a green protocol for recovering betalains from unsold beets as a natural food colorant.

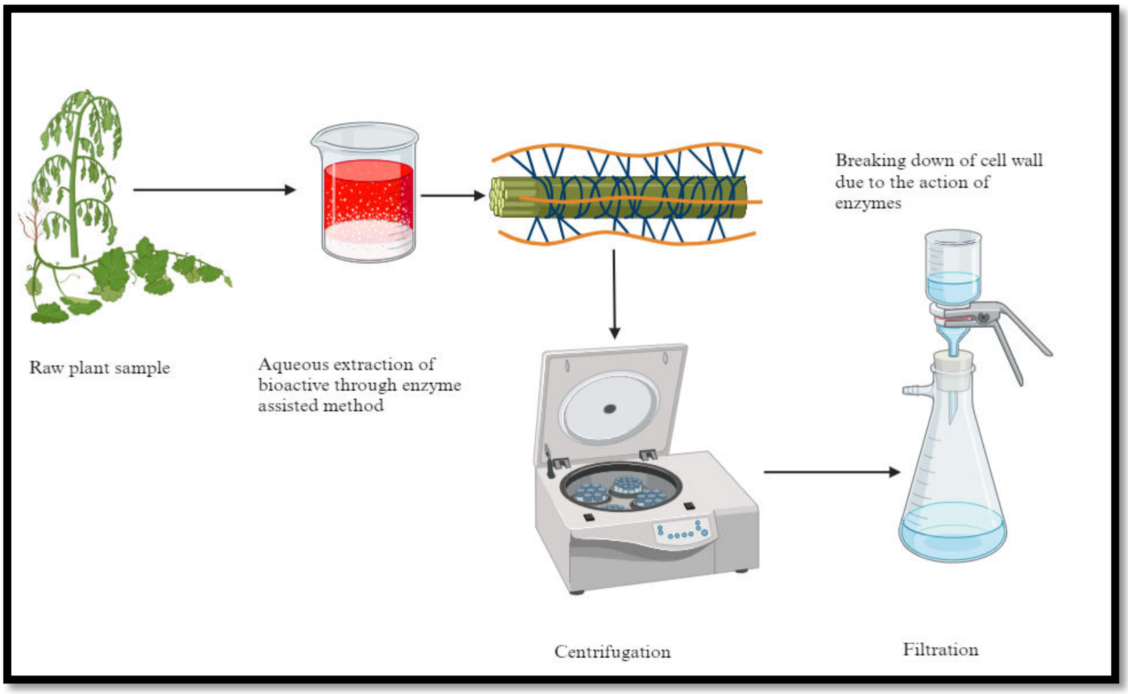


Fig. 12 Enzyme extraction process

4.2.6 Microwave-assisted extraction(MAE)

Plant cell walls can rupture due to the rapid expansion of plant cell structures caused by microwave heating. This phenomenon allows substances, including pigments, to easily diffuse out of the cells, thereby accelerating the extraction process (Zou et al., 2013). For example, Dabiri et al. (2005) demonstrated that microwave-assisted extraction (MAE) can reduce the extraction time and solvent requirements for extracting pigments (such as alizarin and purpurin) from Rubiaceae plants. Compared to Soxhlet extraction, which required up to 360 minutes and 100 milliliters of solvent, MAE only required 20 minutes and 20 millilitres of solvent. Additionally, MAE performed under optimal conditions resulted in higher recovery of alizarin and purpurin compared to Soxhlet extraction under ideal conditions.

Mechanism of MAE- Dipole rotationand rotation-Plant materials contain polar molecules (e.g., water, pigments, and other constituents) and ionic species. When exposed to microwave radiation, these polar molecules and ions try to align themselves with the oscillating electromagnetic field, resulting in rotational motion and migration, respectively. This molecular movement generates heat through molecular friction and ionic conduction, leading to a rapid increase in temperature. Cell disruption: The rapid heating caused by microwave energy disrupts the plant cell walls and membranes, facilitating the release of intracellular compounds into the surrounding solvent. This disruption occurs due to the rapid expansion of water molecules inside the plant cells, creating a pressure buildup that eventually ruptures the cell walls. Solvent heating and improved mass transfer: In addition to heating the plant material, microwave energy also heats the surrounding solvent, increasing its ability to dissolve and extract the desired compounds.

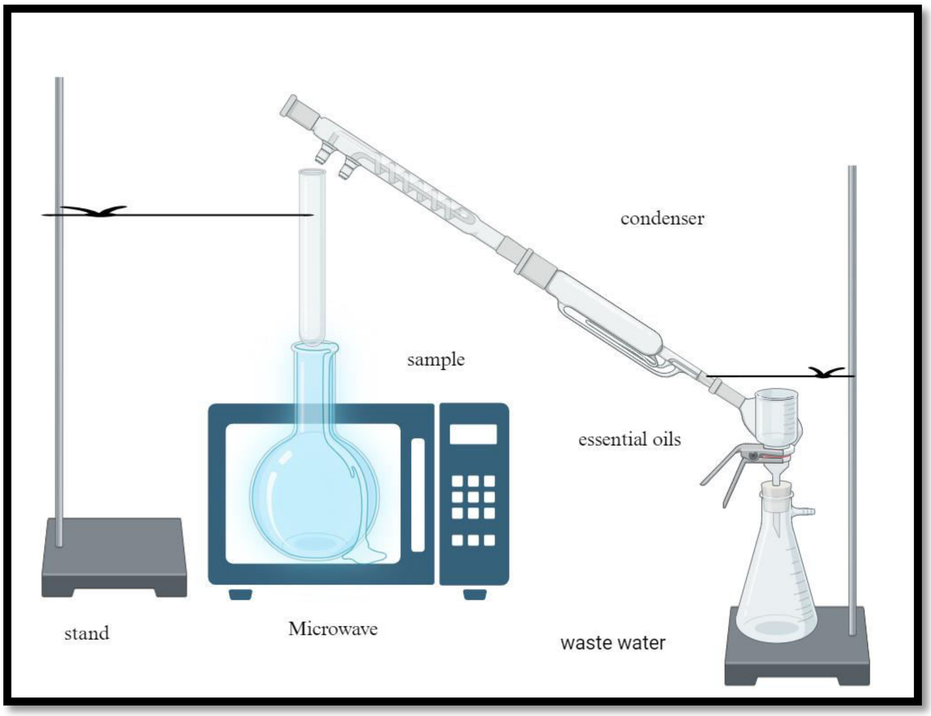


Fig. 13 Microwave assisted extraction

Ugarte et al. (2014) investigated the use of microwave-assisted extraction (MAE) to extract betalains (betanines and betaxanthins) from diced fruits and vegetables, with a primary focus on beetroot. The study tested different combinations of microwave power, duty cycle, and time. The highest betanine yield was achieved by applying 400W at 100% duty cycle for 90-120 seconds, while the highest betaxanthin yield was obtained at 140-150 seconds. The addition of ascorbic acid to the solvent and using a two-step MAE process with a cooling period in between were found to enhance the pigment yields. Overall, MAE resulted in betalain yields twice as high as those obtained with conventional extraction methods.

Table 3- Biocolors obtained from different extraction technologies

Extraction method	Target compound	Extraction	Advantages	References
SFE(Super Critical Field Extraction)	Anthocyanin from Haskap Berry (Lonicera caerulea) pulp	Total anthocyanin yield (52.7%), using CO2, 45 MPA pressure	Compared to other techniques, SFE enables the extraction of chemicals at comparatively low pressures and temperatures. This benefit allows for the preservation of heat-sensitive components' integrity, which makes it appropriate for the non-degradation extraction of delicate natural substances like flavours and essential oils.	Jiao et al., (2018)
PEF(Pulse Electric Field)	Betalains (red beet, Beta vulgaris L.)	Extract recovered 95%	PEF technology improves the extraction of intracellular chemicals by permeabilizing cell membranes with short electric pulses. This non-thermal technique is very helpful for removing bioactive chemicals from plant materials and microbial cells while maintaining the integrity of heat-sensitive materials.	Loginova et al. (2011)
Microwave assisted extraction (MAE)	Anthocyanins (Crocus sativus flower's tepal)	101.0 mg/g which is about 77% of total monomeric anthocyanin was extracted	MAE heats both the sample and the extraction solvent directly using microwave energy. Rapid heating that is targeted at breaking down cell walls and liberating intracellular components facilitates effective extraction. One of MAE's main benefits is its capacity to drastically cut down on extraction times, which makes it a time-efficient technique for extracting a variety of compounds.	Jafari et al. (2019)
MAE	Phycobiliprotein from porphyridium purpureum	Extraction time was reduced by nearly 180–1080 folds and pigment purity up to 3.8-fold		Juin et al. (2015)
Enzyme aided	Lycopenewas extracted using	Yield increased by 8–18 folds	Utilising the specificity of enzymes, enzyme-aided extraction	Zuorro et al. (2011)

extraction	cellulose: Pectinase in the ratio of 1:1		improves the release of valuable chemicals from biological materials by dissolving cell walls. This technique preserves delicate bioactive compounds by working in mild settings.	
HPE	a-carotene,b-carotene & lutein from grapes and oranges	Yield obtained were 38.06%, 53.78% and 361.17% respectively	High temperatures and pressures can be effectively extracted using high-pressure extraction methods, such as PLE. This approach shortens the extraction time and increases overall process efficiency, making it useful for extracting a variety of chemicals, such as flavours, perfumes, and bioactive substances.	Khan et al., (2018)

5.0 Effect of different components in extractionof betalains

The use of betalains as natural colorants can offer benefits due to their associated health properties and safety. However, substituting synthetic food dyes with betalains can be challenging. An important consideration is extracting betalains from the plant matrix, which should aim for maximum yield to supply sufficient material for industrial applications. A challenge with powdered colorant extracts from fruits and vegetables is the presence of compounds other than the pigment of interest, which can affect the quality of the food product they are applied to, as is the case with beetroot. The main issue related to using betalains as natural colorants is their limited stability during processing and storage, which will ultimately impact the color of the food product. Studies have shown that the thermal degradation of beetroot betalains in milk follows first-order reaction kinetics, meaning higher temperatures lead to increased degradation.

5.1Effect of pH on the exctraction process

pH plays a crucial role in the extraction process of betalains, as it can affect the stability of the emulsion and, consequently, its extraction efficiency. Neagu and Barbu (2014) observed that pH has a positive effect on the extraction process when carried out at low temperatures (20°C) compared to a temperature of 70°C, at which pH has no significant effect. Das et al. (2019) noted an increase in betalain extraction yield from red amaranthus (Amaranthus cruentus) with acidification from a neutral medium to pH 5. Similar results were observed with red beetroot (Beta vulgaris L.) by Pandey et al. (2013). Therefore, it is recommended to acidify the extraction medium to enhance and facilitate the accumulation of betalains during their extraction (Mohammed et al., 2018). Citric acid is commonly used as an acidifying agent because it acts as a stabilizing agent for the electrophilic center of betalains, thus improving their stability (Prakash-Maran et al., 2013).

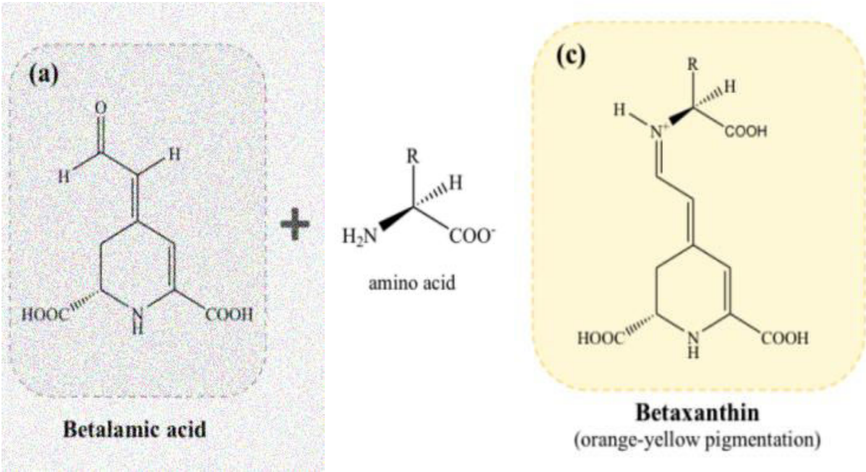
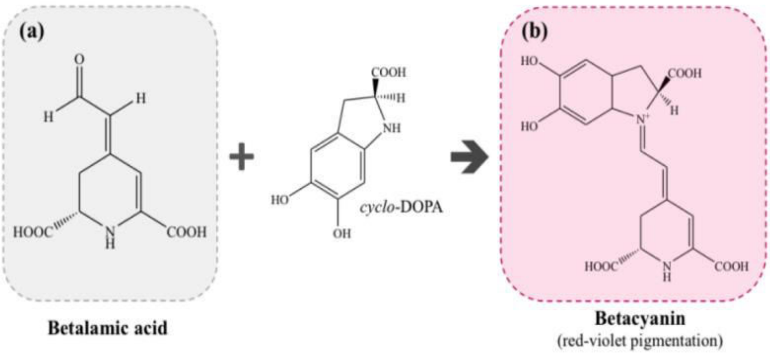


Fig.14 (a) Betalamic acid, the basic structure of the betalains and (c) general structure of the betaxanthines derived from the condensation of the betalamic acid with amino acids or its derivatives.



(b) general structure of the Betacyanin's derived from the condensation of the betalamic acid with cyclo-DOPA;

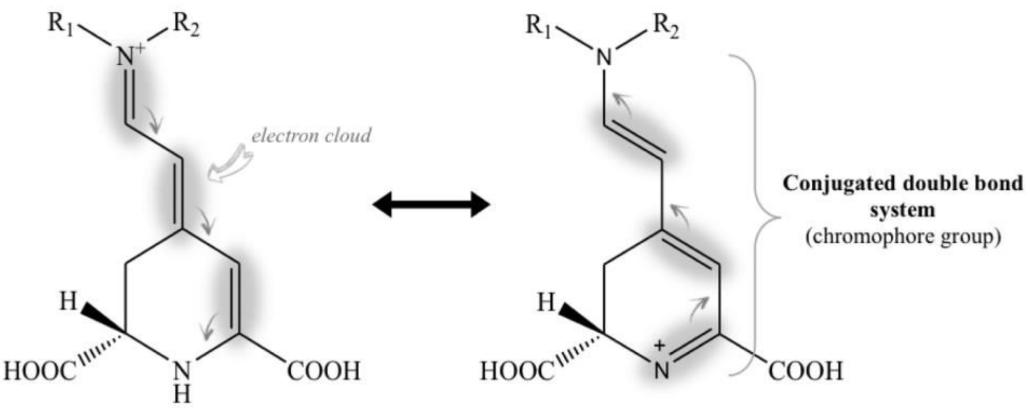


Fig. 15. Resonance structure of betalain. The gray shade represents the electron cloud within the conjugated double bond system, and the gray arrows indicate the conjugate displacement of electron cloud

Table 4-Different samples and their extraction methods

Sample	Extraction Method
Flowers/Bracts **Amaranthus Spp.** **Bougainvillea Spectabilis** **Fruits** **Basells Rubra L.**	Solid-liquid extraction Solid-liquid extraction Maceration Solid-Liquid extraction Ultrasound-assisted (UAE) extraction
Prickly Pear (Optunia Ficus Indica) **Prickly Pear (Opunitia Spp.)**	Solid-extraction High pressure Carbon Dioxide (HPCD) High-Pulsed Electric Fields (HPEF)
Fruit Peels **Opuntia Engelmannii** **Red Dragon Fruit (Hydrocereus Polyrhizus)**	Ultrasound-assisted extraction Solid-Liquid extraction
Leaves **Amaranthus Spp.** **Red Amaranth (Amaranthus Cruentus)**	Solid- liquid extraction Solid- liquid extraction
Roots **Grown Red and Golden Beets (Beta Vulgaris L.)** **Red Beetroot (Beta Vulgaris L.)**	Ultrasound-assisted extraction (UAE) Ultrasound-assisted extraction (UAE) Solid- liquid extraction
Whole Plant **Alternanthera Sessilis**	Solid-liquid extraction

6.0 Encapsulation of betalains

Encapsulation is a process that involves enclosing solids, liquids, or gaseous substances in small capsules, which can be either nanometric or micrometric in size (Fang and Bhandari, 2010). The contents of these capsules are isolated from the surroundings and can be released at a controlled rate over an extended period by various triggers such as pH, enzymes, and temperature (Augustin and Hemar, 2009; Gouin, 2004). This technology is particularly significant in the medical field for controlled drug release and vaccine delivery. It is also valuable in the food industry for incorporating functional ingredients such as antioxidants and antimicrobials, as well as for controlling flavor, texture, and color (Champagne and Faustier, 2007; Winskovic et al., 2017).

Encapsulation is based on protecting sensitive compounds from external factors by enveloping them in a more stable matrix, such as polysaccharides, lipids, and proteins. These systems are often composed of core-shell structures, where the protective emulsion is the core, and the shell or matrix surrounds the emulsion (Janiszewska, 2014).In a specific study, red beet betalains extract was encapsulated using maltodextrin DE (Dextrose Equivalent) 20 as the main carrier (control). Other polysaccharides, including guar gum, gum arabic, pectin, and xanthan gum, were also tested as encapsulating agents in combination with maltodextrin.

6.1 Methods of encapsulation-

The encapsulation of betalains was prepared by first mixing 100 ml of the extract with 20 g of maltodextrin DE 20, ensuring a homogeneous mixture, which served as the control. To evaluate the encapsulating agents—gum Arabic, pectin, guar gum (GG1), and xanthan (xan1)—xanthan gum was partially replaced instead of maltodextrin by 1 g. For the variations with guar gum (GG2) and xanthan (xan2), approximately 0.5 g was used. The carriers were thoroughly dry mixed before being combined with the liquid extract. The mixtures were continuously and thoroughly stirred to ensure homogeneity during the drying process.

6.1.1Spray drying

Process included dispersion of the core material in an entrapment material, followed by atomization and spraying of the mixture in a hot air desiccant into a chamber. The encapsulated mixtures were spray dried. The spray dryer was operated at inlet temperature ranging from 90 to 102 ° C. The air flow was 700 l/h, rate of feeding 10 ml/min and atomization pressure 25 psi. Encapsulated dry food color powder was obtained as end product.

Segovia et al. (2021) investigated the effect of adding pea protein as an encapsulating agent to beetroot juice, and the spray-drying temperature on the physicochemical, structural, and functional properties of the resulting powder. Spray drying was conducted at 125°C and 150°C with 3.5% and 7% pea protein mixed with the beetroot juice. Various characteristics of the obtained powder were analyzed, including water content, bulk density, porosity, hygroscopicity, water solubility, water absorption index, color, microstructure, betacyanin, total phenols, antioxidant capacity, and powder encapsulation efficiency.Study found that incorporating pea protein in the spray drying of beetroot juice yielded high spray drying yields and desirable powder characteristics. The powder with 7% pea protein was more porous, luminous, and less hygroscopic than the one with 3.5% pea protein. However, 7% pea protein increased water immobilization and reduced soluble solids in the product compared to 3.5% pea protein. The 7% pea protein better protected beetroot bioactive compounds than 3.5%. A higher spray-drying temperature of 150°C significantly decreased the phenol content and antioxidant capacity of the beetroot powders. The optimal conditions for retaining bioactive compounds and antioxidant capacity were 7% pea protein mixed with beetroot juice and a spray-drying temperature of 125°C. This formulation also exhibited higher encapsulation efficiency, resulting in a more functionally stable powder.

6.1.2 Freeze drying

The samples were frozen for 3hat −18 ° C. They were subsequently freeze dried for 2 days to ensure complete drying (freeze drier Alfa-Christ, Germany). The final product was in dry powder form. The encapsulated pigment powders were stored in 50 ml plastic airtight containers sealed with screw caps until further used and analysis.

Ravichandran et al. (2012) In their study, betalains were extracted from red beets and encapsulated using different carrier agents, followed by freeze-drying or spray-drying. The effect of various encapsulating agents, such as maltodextrin, guar gum, gum Arabic, pectin, and xanthan gum, at different concentrations, was evaluated on betalain stability. Encapsulation with a combination of xanthan gum and maltodextrin resulted in approximately 65% higher betalain recovery compared to the control. Freeze-drying yielded a 1.3 times higher recovery of encapsulated betalains than spray-drying. Spray-dried samples exhibited higher lightness (L*) values than freeze-dried samples. The freeze-dried samples containing maltodextrin with xanthan gum and guar gum exhibited the highest chroma value of 21. Stabilizing pure betalain pigments may promote their use as coloring agents in the food industry and foster their broader application.

6.1.3 Ionic gelation-

Ionic gelation is a technique used for encapsulating bioactive compounds, particularly hydrophobic ones, by combining a polyelectrolyte with a multivalent ion of opposite charge to form hydrogel particles. This method is advantageous because it does not require high temperatures or solvents, making it easy to implement on a laboratory or industrial scale. However, encapsulating hydrophilic compounds using this technique can be challenging due to their miscibility with the coating agents, leading to potential compound losses in the cross-linking solution and during storage.To address these challenges, emulsion can be used as a pretreatment, and a combination of polymers can be employed. For instance, betaxanthin encapsulated by spray drying showed higher stability in high humidity environments compared to ionic gelation.

Otálora et al. (2015) performed betalain encapsulation using ionic gelation as a stabilization strategy for these natural pigments. Betalains were extracted from purple cactus fruits and encapsulated in calcium-alginate and a combination of calcium alginate and bovine serum albumin. The beads were characterized by various techniques, including scanning electron microscopy, differential scanning calorimetry, and thermogravimetric analysis. Moisture sorption isotherms were determined, and pigment storage stability was evaluated at different equilibrium relative humidity and temperatures. Study found that the bead morphology was influenced by the matrix composition. Pigment composition of the beads was determined by HPLC-MS-MS, and degradation products, with betalamic acid being the major one, were analysed after storage. Both matrices protected the encapsulated pigments, with better storage stability observed at low relative humidity compared to the non-encapsulated control. The antiradical activities of the beads were proportional to the remaining betalain contents. However, at high relative humidity, no protection was observed, and low storage stability was evident in the samples.

6.1.4 Complex coacervation

Complex coacervation is a technique used to encapsulate bioactive compounds by interacting oppositely charged polyelectrolytes in an aqueous form. It can involve a single polymer or two or more oppositely charged polymers, leading to coacervate formation and phase separation. This method is commonly used to encapsulate lipophilic materials like carotenoids.

Namazadeh et al. (2022) utilized the coacervation technique to encapsulate bioactive compounds from red beets. They used a hydroethanolic solvent to obtain the red beet extract, which was then encapsulated in a complex of positively charged chitosan and negatively charged Persian gum. The optimal condition for coacervate formation was achieved at a specific chitosan to Persian gum ratio at a certain pH. The study found that the coacervation yield of the two polysaccharides was high, and the zeta potential of the dispersion was close to zero. Complex formation between the two biopolymers was confirmed through FTIR analysis. The micro-sized coacervates exhibited high encapsulation efficiency for a specific betalain extract to coacervate dispersion ratio. The betalain-loaded chitosan/Persian gum complex demonstrated superior thermal stability compared to the pure polysaccharides. The obtained complex can be applied to improve the stability of betalains and control their release in food and pharmaceutical applications.

6.1.5 Nanoprecipitation

Researchers employed a nanoprecipitation technique based on two phases, a solvent and a non-solvent phase, under mild magnetic stirring. This technique typically utilizes polymers, particularly bio-based polyesters and starch. The nanoparticle suspension in water can be achieved through methods like solvent evaporation at mild temperature or ultracentrifugation and freeze-drying. Nanoprecipitation is considered a low-cost method with high efficiency and good reproducibility.

Hien-Tran et al. (2023) encapsulated hydrophilic betalains from a red beetroot extract through double emulsification. They prepared a primary water-in-oil (W/O) emulsion using a hydrophobic surfactant for a specific oil. This emulsion was created by combining magnetic stirring and ultrasonication. The resulting double nanoemulsions of betalains (DNB) were characterized to determine the mean particle size using dynamic light scattering (DLS) and transmission electron microscopy (TEM) imaging. The study found that the DNB exhibited color fastness and stable particle size within a specific pH range during storage stability evaluation

Table-5-Effect of processing factors on the stability of betalains obtained from different food sources

Process	**Condition**	**Products**	**Main findings**	**Reference**
Storage Temperature and Light	25,35 and 45°C and light with/without aluminium foil	Red beet juice	Degradation of betalains change in total phenols and color	Kayın et al.,2019
Heating	70-90 °C	Beet root	Degradation of betalains and colorparameters	Güneş̧er,2016
Thermal Stability and Ultra Sound Treatment	0-80°C	Colored quinoa (chenopodium quinoa wild) hulls	Thermalstabilitywassimilartothatofbetalainsfrombeetroot	Laqui-Vilca et al.,2018
High Pressure Processing (HPP) And High Temperature Short Time (HTST) Thermal Treatment	HPPwasappliedat00barfor10,20and30minandHTSTtreatmentwasapplied at75.7°Cfor80s,81.1°Cfor100sand 85.7°Cfor120s	Red beet stalks	HPPtreatmentdidnotshowanyimprovementinthebetalainstability. HTSTwasconsideredthemostsuitabletomaintainbetalainstabilityfromredbeet.	dosSantoset al.,2018
Technological Process	Boiling, Roasting, Microwaving and Vacuuming	Red beet	Vacuumandmicrowaveproduceincreasesinbetalains,whileboilingandroastingproducesadecrease	Ravichandranet al.,2013

Food Additives and Ph	Ascorbic and citric acid at pH 4 and 6	Yellow-Orange cactus pear	Pigmentstabilityandcolorcharacteristicsdependedontypeandconcentrationoftherespectiveadditiveaswellason pHconditions.	Moßhammer et al.,2007
Technological Processes And In Vitro Digestion	Boiling, microwave and fermentation	Red beet products	Technological processes reduced the content of betalain by 42-70% in the obtained products. The contribution of betalains released from red beet products after an in vitro digestion was detected within the range of 0.001-0.10%.	Sawickiet al.,2017

7.0 Application of betalains in food industry

Recent applications in food have shown that betalains can be applicable to various food products as natural colorants and also in drink and functional food formulations (Panghal et al., 2017). These natural pigments provide violet to yellow colors, which have applications in desserts, ice creams, jams, candy, jellies, soups, sauces, drinks, and cow milk (Jurić et al., 2020). Fruits containing betalains are also made into various commercial products such as juices, dehydrated fruits, and jams.

Studies have evaluated that three factors are highly considerable during the incorporation of natural colorants like betalains into food products: the betalain source and profile, storage conditions, and food matrix composition. These factors play a crucial role in determining the stability and performance of betalains in food applications.

Table 6 - Betalains utilization in food

Sources	Color of pigment	Method of extraction	Method of encapsulation	Applicationin food industry	End quality	References
Food applications						
Beetroot	Red	Ultrasound	Vaccum drying	Cookies	Betalain-incorporated cookies showed	Chaudhary et al. (2021)

					maximum acceptability with moisture content (3.75%), ash (1.25%), protein (14.21%), carbohydrate (73.29%), total energy (418 Kcal), and fat (7.5%).	
Beetroot powder	Red	Microwave assisted	Spray drying	Yogurt	Yogurt with the incorporation of beetroot powder showed pH 4.56, TA 7.39 g lactic acid/100 g, syneresis 43.60%, and water holding capacity 40.64	Dabija et al. (2019)
Beetroot powder	Red	Ultrasound	Domestic blender	Noodles	Adding 15% beetroot powder to 85% wheat flour significantly increased the a* value (0.75 to 5.79), protein content (12.66% to 13.37%), total ash content (1.24% to 1.99%), crude fiber content (2.09% to 2.38%), carbohydrate	Abiodun et al. (2020)

					content (70.81% to 72.34%), DPPH antioxidant activity (37%), aroma (5.60 to 5.65), and overall antioxidant potential (39.22%).	
Beetroot extract	Red	Microwave	Freeze drying	Sausage	Sausages containing beetroot extract powder had 26.77% moisture, a pH of 5.85, and 19.03 μmol MDA/kg TBARS. The color values were 47.27 for L*, 23.72 for a*, and 17.68 for b*. The appearance, color, odor, flavor, texture, and overall acceptance scores were 7.00, 7.18, 5.33, 6.45, 5.65, and 6.43, respectively.	Aykln-Dincer et al. (2021)
Cactus Pear (Opuntia ficus- indica)	Yellow	Ultrasound	Spray drying	Yogurt	Cactus with maltodextrin retained Indicaxanthin (83.4%), color (0.61) in	Carmona et al. (2021)

					yogurts, pH was 4.21–4.23 at 4 ◦C after 27 days	
Stenocereus Pruinosus fruits	Yellow	Microwave	Solution form	Gummies and beverages	Beverages pigmented with betaxanthin resulted with h◦ 64.6–87.1, L* 44.0–68.9, a* 1.7–35.2, b* 32.6–82.2, 5.4–85.2.	Rodriguez-Sanchez et al. (2019)
Basellace fruit	Violet red	Ultrasound	Solution form	Gummy candies	After 28 days, there was a decrease in total soluble solids (TSS, 70.67 °Brix), lightness (L*, 12.78), yellowness (b*, -0.48), chroma (12.35), hue angle (-0.04), water activity (0.72), cohesiveness (0.10), gumminess (226 g), and springiness (7.22 g), but an increase in hardness (1945 g) and stiffness (818 g/mm). No microbial contamination was detected.	Sravan Kumar et al. (2020)
Cactus fruit pulp	Purple	Lyophilizer	Lyophilization	Gummies candies	L* (22.50), b* (2.50)	Otalora et al.

					increased while a* (20.50) decreased after 30 days at 4 ◦C	(2019)
Red pitaya pulp	Magenta purple	Microwave	Spray drying	Yogurt	In yogurt with betalain, 10% maltodextrin, and 1% mucilage stored at 5°C for 30 days, the maximum values were observed for L* (67.87 ± 0.01), chroma (12.50 ± 0.02), and hue angle (8.30 ± 0.03).	Utpott et al. (2020)
Red beet (B. vulgaris L.)	Red	Ultrasound	Solution form	Gummy candies	Gummycandies containing liposomes showed higher betanin (89.95 and 77.16%) and DPPH inhibition (85.09 and 82.28%) retention over 60 days than free candies and overall acceptance (5.8) detected in free betanin (10%) candies	Amjadi et al. (2018)
Non-Food Applications						

Red Amaranth (A. tricolor L.)	Red	Solvent extraction	Freeze drying	Packaging film	Film that is incorporated with beetroot powder about 80g of fresh shrimp were placed in a 12cm x 12cm x 4cm polypropylene box with a lid. The shrimp were stored at 20°C for 48 hours. Total volatile basic nitrogen (TVB-N) levels and color changes of the shrimp were measured every 8 hours during storage.	Yao et al. (2021)
Cactus pear (Opuntia ficus-indica, 'Gialla')	Green	Fine chopping and cutting manually	Freeze drying	Edible coating	CO_2 progressively increased over storage in each treatment, with average values ranging between 5.0 and 5.7 kPa after 12 days of storage, while the corresponding in-package O_2 partial pressure never dropped below 14 kPa.	Palma et al. (2019)

Prickly pear fruit Opuntia basilaris P.	Yellow	Solvent extraction	Freeze drying	Edible coating	Betacyanins were individually isolated from red pitaya, prickly pear, red beetroot, globe amaranth and red amaranth. The total betacyanin contents in the RPFE, PPFE, RBRE, GAFE and RALE were 48.22, 104.33, 50.95, 27.50 and 44.42 mg betanin equivalents/g lyophilized extract, respectively	Xiyu Yao et al. (2021)
Paper flower (Bougainvillea glabra)	Red	Solvnet extraction	Lyophilization	Edible film	After addition of betacynanins the freshness of shrimp and fish freshness indicator having 48mg/240mL biopolymer solution adding TiO_2 NP_s	Yao et al. (2022)
Bougainville a glabra Choisy flowers	Yellow	Fine chopping and cutting	Freeze drying	Edible coating	A starch film containing 15% betacyanin was capable of visually detecting the changes in	Naghdi S et al. (2021)

					Caspian sprat quality during cold storage by undergoing a color change from pink to yellow, coinciding with microbiological and chemical alterations in the fish samples.	

Various drying and encapsulation techniques have been developed to produce stable forms of betalains. Dried beetroot is utilized in edible coatings and cheese products. Red beetroot juice containing betalains is used in pasta and chicken frankfurters. Red beet chips are also available as a fried snack option. Prickly pear is incorporated into cereal-based products. Betacyanin pigments are studied for their impact on wheat-based foods to improve quality and expand their applications (Msaddak et al., 2017).

Researchers have demonstrated a direct relationship between the betalain content and antioxidant capacity of Amaranthus spp., measured using the Fluorescence Recovery After Photobleaching (FRAP) method. It was found that leaves exhibit higher antioxidant activity compared to other parts of the plant. Aerial parts, leaves, and fruit peels are commercial sources of betalains. In confectionery, betalain applications are limited, with examples being the use of red pitaya fruit puree pigments in gummy production with gelatin and pectin, beetroot powders and pitaya juice as natural pigments in candy formulations, and betanin-nanoliposomes in gummy candy formulations (Amjadi et al., 2018).

Roriz et al., (2018) have extensively studied the use of betalains as colorants, antioxidants, and antimicrobials. The acceptability of betalain-incorporated products has been found to depend on the betalain concentration and source. Betalains have been used as natural color pigments in ice creams, increasing product stability for up to 180 days at 20°C (Roriz et al., 2018). They have also been used as natural colorants in fruit spreads, banana juice, and cow milk, with their stability and acceptability depending on factors such as storage temperature, thermal treatments, and the betalain source and profile.Betalains have also been explored for their antioxidant properties in various foods. Their incorporation has been shown to inhibit microbial growth and improve sensory perception of products. Betalains from sources like pear fruits have demonstrated protective effects on dairy products, reducing oxidation in yogurt and ice cream.

While pokeberry juice was initially used to enhance color in food products, its use was banned due to the presence of purgatory and emetic saponins. However, further research revealed that the coloring compound in pokeberry juice is identical to betanin found in red beets. Betacyanin from O. stricta is now valued for its flavor and used in yogurt and soft drinks.The profile of betalains determines their application in short-shelf-life foods produced with moderate thermal treatment, as well as products marketed in dry conditions with low light and humidity. Betalains are used in a variety of food products, including desserts, poultry, dairy, and meat products.

8.0 Challenges and Future Prospects

Certain challenges such as stability, cost-effectiveness, and scalability need to be addressed. This section discusses ongoing research and technological advancements aimed at overcoming these challenges. There are limitations to using natural pigments, such as limited availability and low concentration. Some sources are seasonal, and quality can vary based on origin. Natural pigments are also sensitive to factors like pH, heat, and light. To address these limitations, microbes can be created using recombinant DNA techniques to produce natural pigments. This allows for controlled growth environments, overcoming seasonal availability issues. Minimal processing reduces costs, and pigments can be added at later stages. Proper knowledge of pigment application, extraction, preservation, and packaging is essential to minimize undesirable changes.

New trends in applying betalains in the food industry involve exploiting the pH-sensitive property of betacyanins. Betalains-rich extracts from sources like beetroot and red pitaya have been incorporated into films and packaging materials. These films can change color in response to pH changes, indicating potential spoilage or loss of freshness in protein-rich foods like fish fillets, shrimp, and animal feed. The color change is triggered by the release of ammonia during protein degradation, causing the betalain-containing films to shift from red/purple to green/orange.

However, the effectiveness of this color change as an indicator of freshness can be limited, and factors such as betalain content in the formulation may negatively impact the color change. Nevertheless, these intelligent packaging materials incorporating betalains offer promising applications by combining antioxidant, antimicrobial, and pH-sensitive properties. Innovation and development in this area are projected to be significant, particularly for the seafood industry, where betalain-based films could serve as smart packaging to monitor freshness and quality.

9.0 Conclusions

The exploration of bio colorants from plant sources represents a promising avenue for enhancing the aesthetics and health profile of foods. This innovative approach aligns with current consumer preferences, regulatory trends, and the broader movement towards sustainable and natural solutions in the food industry. As research and development in this field continue, the potential for creating visually stunning and nutritionally rich food products using bio colorants remains an exciting prospect. Bio colorants represent a viable and eco-friendly alternative to synthetic colorants. Their diverse sources, extraction methods, applications, and sustainability benefits make them a compelling option for industries aiming to reduce their environmental impact. Continued research and development in this field are crucial for unlocking the full potential of bio colorants and ushering in a more sustainable era.

While betalains are primarily valued for their coloring properties in the food industry, their potential health effects require further research. The use of betalains in food often involves a trade-off between their aesthetic appeal and potential health risks, as some betalain compounds may cause adverse effects. However, it is essential to note that reports on the health effects of fruits or juices containing betalains may be influenced by the presence of other non-betalain components, leading to biased results.

Betalains shows potential for development into antimicrobial drugs, but more research is needed. Natural foods are popular due to their nutrient content, and there's a growing trend for healthy diets with natural additives. Thermal processing affects the color and flavor of food, making the addition of food additives necessary to improve these properties. Food color is important for freshness and consumer appeal. Artificial pigments are limited due to their association with chronic diseases. Natural pigments are preferred because of their health benefits and lower toxicity concerns. However, challenges like cost, efficiency, and stability are associated with their application.

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