Bioactive Substances Applied in the Food Industry – Anthocyanins

Food Science

This course is intended for students pursuing a Master's degree in **Product Quality and Food Safety**, **Applied Microbiology**, and **Microbial Biotechnology**.

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Foreword

Anthocyanins constitute a major class of natural pigments belonging to the family of phenolic compounds. They are widely distributed in the plant kingdom and are responsible for the red, purple, and blue colors observed in numerous fruits, vegetables, flowers, and processed food products. Beyond their coloring role, anthocyanins have attracted increasing scientific interest due to their distinctive chemical and biochemical properties, their sensitivity to processing and storage conditions, and their beneficial biological effects on human health.

In a context marked by growing requirements for food quality, food safety, and the valorization of natural compounds, anthocyanins occupy a strategic position in the agri-food sector as well as in the fields of nutrition, biotechnology, and health sciences. Their involvement in food sensory quality, their therapeutic potential, and their application as natural colorants make them compounds of significant scientific and industrial relevance.
This course handout, entitled **"Anthocyanins"**, is primarily intended for **Master's students** specializing in:

- **Quality of Products and Food Safety**;
- **Applied Microbiology ;**
- **Microbial Biotechnology**.

Its main objective is to provide students with solid and up-to-date theoretical foundations necessary for understanding the role of anthocyanins in food systems, biological processes, and technological applications, while establishing clear links between fundamental concepts and practical uses.

The handout is structured into six complementary chapters. The first chapter focuses on the **chemistry and biochemistry of anthocyanins**, including their structure, classification, and main properties. The second chapter addresses the **stability of anthocyanins**, highlighting the influence of physicochemical factors such as pH, temperature, light, and oxygen. The third chapter examines **anthocyanins in foods**, in relation to nutritional quality

and sensory attributes. The fourth chapter is devoted to the **therapeutic aspects of anthocyanins**, emphasizing their biological activities, particularly their antioxidant and protective effects. The fifth chapter presents the **biosynthesis of anthocyanins**, describing the metabolic pathways and enzymatic mechanisms involved. Finally, the sixth chapter discusses the **use of anthocyanins as food colorants**, as natural alternatives to synthetic colorants.

This handout has been designed as a pedagogical tool to support learning, revision, and knowledge enhancement. It aims to accompany students throughout their academic training, to strengthen their scientific analytical skills, and to serve as a reference basis for research activities and professional applications in the fields of food quality, food safety, and biotechnology.

Acknowledgements

The author wishes to express sincere gratitude to **Professor OUCHEMOUKH Salim** and **Dr. ADJAOUD Kahina** for having agreed to contribute to the scientific review, expert evaluation, and final revision of this document. Their insightful comments, constructive suggestions, and careful reading will contribute significantly to improving the scientific rigor, clarity, and overall quality of this course handout. Their expertise, dedication, and commitment to academic excellence will be instrumental in the completion of the final version of this work.

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Abbreviations

ABA: Abscisic acid
ABC: ATP-Binding Cassette
ABCG1: ATP-Binding Cassette Transporter G1
ABTS$^{+\bullet}$: 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical
AD: Alzheimer's Disease
ALS: Amyotrophic Lateral Sclerosis
ANS: Anthocyanidin Synthase
Apo: Apolipoprotein
Aβ: Amyloid Beta
CCL2: C-C Motif Chemokine Ligand 2
CCR2: CCL2 Receptor
CD33: Immunoglobulin-like Microglial-Surface
CHI: Chalcone Isomerase
CHS: Chalcone Synthase
CVDs: Cardiovascular Diseases
CX3CR1: C-X3-C Motif Chemokine Receptor 1
DFR: Dihydroflavonol Reductase
DPPH$^{\bullet}$: 2,2-Diphenyl-1-picrylhydrazyl Radical
FRAP: Ferric Reducing/Antioxidant Power
F3H: Flavanone-3-Hydroxylase
F3JH: Flavonoid-3'-Hydroxylase
FT-IR: Fourier Transform Infrared Spectroscopy
GSK3: Glycogen Synthase Kinase 3
HAT: Hydrogen Atom Transfer
HD: Huntington's Disease
HDL: High-Density Lipoprotein
HO-1: Heme Oxygenase-1
HPLC: High-Performance Liquid Chromatography
HR-MS/MSn: High Resolution and Tandem Mass Spectrometry
HSCCC: High-Speed Counter-Current Chromatography
iNOS: Inducible Nitric Oxide Synthase
ICAM-1: Intercellular Adhesion Molecule-1
IL: Interleukin
JNK: c-Jun-N-terminal Kinase
7-KC: 7-Ketocholesterol
LDL: Low-Density Lipoprotein
LPS: Lipopolysaccharide
MDA: Malondialdehyde
MCP-1: Monocyte Chemoattractant Protein-1
MMD: Monocyte to Macrophage Differentiation Associated
MPK: MAP Kinase
NADES: Natural Deep Eutectic Solvents
NBT: Nitro Blue Tetrazolium
NMR: Nuclear Magnetic Resonance
Nrf2: Nuclear Factor Erythroid 2–Related Factor 2
oxLDL: Oxidized LDL

OZR: Obese Zucker Rat
PAL: Phenylalanine Ammonia-Lyase
PCA: Protocatechuic Acid
PD: Parkinson's Disease
PEG-AuNPs: Anthocyanin-Loaded Polyethylene Glycol-Gold Nanoparticles
PI3K: Phosphatidyl-Inositol-3-Kinase
PON1: Paraoxonase 1
PPARs: Peroxisome Proliferator-Activated Receptors
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species
SAMP8: Senescence-Accelerated Mice Prone 8
SCFA: Short-Chain Fatty Acids
SET: Single Electron Transfer Mechanism
SPE: Solid Phase Extraction
SPL: Squamosa-Promoter Binding Protein-Like
TC: Total Cholesterol
TG: Triglyceride
TLR2: Toll-Like Receptor 2
TNF-α: Tumor Necrosis Factor Alpha
TREM2: Triggering Receptor Expressed on Myeloid Cells 2
TT8: Transparent Testa 8
TTG1: Transparent Testa Glabra 1
UFGT: UDP-Glucose:Flavonoid-3-O-Glycosyltransferase
UPLC: Ultra-Performance Liquid Chromatography
VCAM-1: Vascular Cell Adhesion Molecule-1
VLDL: Very Low-Density Lipoprotein

Introduction

The rapid increase in global life expectancy, often attributed to healthcare, nutrition, and sanitation improvements, has significantly enhanced the quality of life for many individuals worldwide. However, along with these advancements, there has been a marked increase in the consumption of hypercaloric foods, particularly processed and convenience foods, predominantly in Western countries. This dietary shift has contributed to the growing epidemic of chronic non-communicable diseases (NCDs), including cardiovascular diseases, metabolic disorders, and neurodegenerative diseases. Poor dietary habits, sedentary lifestyles, and environmental factors largely drive these conditions.

In this context, there is a growing emphasis on promoting the consumption of fresh, minimally processed foods that contain bioactive compounds. These compounds, which are not essential nutrients but contribute to the prevention and management of various diseases, offer a wide range of health benefits. Polyphenols are a prime example of such bioactive compounds, a large class of naturally occurring molecules in plant-based foods such as fruits, vegetables, nuts, and seeds. Polyphenols, especially anthocyanins, are particularly interesting due to their potent antioxidant, anti-inflammatory, anticancer, glucose-regulating, and neuroprotective properties. These compounds have the potential to modulate numerous biological pathways and processes, offering protection at both the cellular and systemic levels.

Among the many polyphenolic compounds, anthocyanins are the most studied and biologically significant groups. These compounds are water-soluble pigments primarily found in plant cell vacuoles, contributing to the characteristic red, purple, and blue colors observed in various fruits, vegetables, and flowers. Anthocyanins are widely distributed in the plant kingdom, playing key roles in the plant's interaction with its environment. For instance, anthocyanins are instrumental in attracting pollinators and seed dispersers to flowers and fruits, which is essential for the reproduction and survival of many plant species. This function is achieved by the vivid pigmentation of flower petals and fruit epidermis, which signals the presence of ripe or pollinator-friendly flowers.

In addition to their role in pollination and seed dispersal, anthocyanins also serve protective functions in plants. These pigments are known to absorb light, providing a natural defense against harmful ultraviolet (UV) radiation. They also help plants cope with abiotic stresses such as extreme temperatures and drought. During periods of environmental stress,

anthocyanin synthesis is often upregulated, suggesting their involvement in mitigating oxidative stress and maintaining cellular integrity under adverse conditions.

Anthocyanins are derivatives of anthocyanidins, flavonoid compounds produced through the phenylpropanoid biosynthetic pathway. These pigments are glycosides, meaning they comprisean anthocyanidin aglycone (the non-sugar component) bound to a sugar molecule. The six most common anthocyanidins found in foods are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin. Each differs slightly in their chemical structure, influencing their color properties and biological activities.

The color of anthocyanins is highly pH-dependent. In an acidic environment, they appear red; in a basic (alkaline) environment, they shift to blue. This pH sensitivity is due to the chemical structure of anthocyanins, particularly the hydroxyl groups on the B-ring of the flavonoid structure. These structural components are crucial in determining the stability of anthocyanins. At alkaline pH, anthocyanins are unstable and may degrade into dark brown compounds due to the breakdown of their chemical structure. The presence of metal ions, temperature fluctuations, exposure to light, and oxygen availability can further influence their stability and degrade their color and nutritional value.

Beyond their role in food coloration, anthocyanins have become valuable nutraceutical ingredients due to their extensive health-promoting properties. Numerous studies, including in vitro, animal, and human research, have demonstrated that anthocyanins possess potent antioxidant capabilities, enabling them to neutralize free radicals and reduce oxidative stress in the body. This antioxidant activity is critical in protecting cells from damage caused by oxidative stress, which is linked to the onset and progression of a variety of chronic diseases, including neurodegenerative disorders (e.g., Alzheimer’s and Parkinson's disease), cardiovascular diseases, and metabolic disorders like diabetes.

In addition to their antioxidant properties, anthocyanins have demonstrated anti-inflammatory effects, which are crucial in preventing chronic inflammation—a common underlying factor in many diseases. These compounds can modulate the activity of several enzymes, such as cyclooxygenase (COX) and mitogen-activated protein kinase (MAPK), and influence signaling pathways related to inflammatory cytokines. The anti-inflammatory effects of anthocyanins have led researchers to explore their potential in managing conditions such as arthritis, atherosclerosis, and inflammatory bowel diseases.

Furthermore, anthocyanins support visual health by protecting the retina and enhancing vision, functioning synergistically with other nutrients like vitamin A and carotenoids. Their potential as protective agents for the visual system is particularly important as the global population ages and the prevalence of age-related macular degeneration (AMD) and other visual impairments increases.

The clinical safety of anthocyanins has been well-documented, with no negative side effects observed, even at high doses. This has fueled interest in their use as preventive and therapeutic agents in treating various chronic diseases. Thus, anthocyanins are valuable for their role as natural food colorants and for their substantial contributions to health maintenance and disease prevention.

This book aims to provide a comprehensive overview of the latest research on anthocyanins, focusing on their chemistry, biochemistry, and the vast array of health benefits they offer. By delving into the molecular mechanisms underlying their biological effects, we hope to shed light on the potential applications of anthocyanins in promoting human health, particularly in the prevention and management of chronic diseases. As our understanding of these fascinating pigments deepens, their role in nutrition and health will undoubtedly become more prominent, offering new avenues for the development of functional foods and natural therapeutics.

I. Chemistry and biochemistry of anthocyanins

I-1 Structural determinants of anthocyanins

Anthocyanins are the glycosylated forms of anthocyanidins (aglycones), naturally occurring flavonoids known for their vibrant colors. These compounds are synthesized through a chemical process that involves a flavylium cation backbone, which is hydroxylated at various positions on the molecular structure. These hydroxyl groups generally appear at carbons C3, C5, C6, C7, and C3', C4', and C5', giving rise to a range of different anthocyanidins, each with unique structural and color properties **(Figure 1)**. Despite the presence of an oxonium group in their structure, the flavonoid skeleton retains its typical ring nomenclature, with the charged oxygen atom located on the C-ring of the molecule. This structure is key to anthocyanins' biological activity and stability and their ability to impart color to various plant tissues.

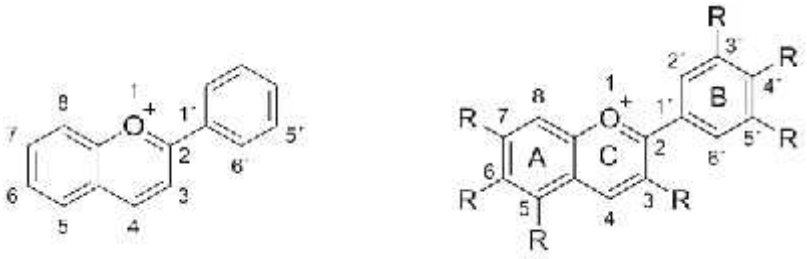

Figure 1. Chemical structure of the flavylium cation on the left and of anthocyanidin backbone on the right with atom numbering and ring label (R = H, OH)

I-1.1 Chemical structure of anthocyanins and anthocyanidins

The structure of anthocyanins is based on the flavylium cation, a positively charged compound that serves as the core of the molecule. The hydroxylation of different positions on the flavylium backbone leads to the formation of anthocyanidins, each having distinct properties and contributing to the color of the plant. The charged oxygen atom on the C-ring plays a critical role in the molecule's stability and color behavior, making it highly sensitive to pH changes and other chemical elements in the environment.

When anthocyanidins undergo glycosylation, they form anthocyanins. Glycosylation refers to the attachment of sugar molecules to the hydroxyl groups of anthocyanidins. The most

common glycosylation site in nature is the 3-hydroxyl group (3-OH), where sugar molecules, such as glucose, are added, resulting in the formation of 3-O-β-glucosides (e.g., chrysanthemin from cyanidin) **(Figure 2)**. This process is catalyzed by the enzyme glucosyltransferase, which transfers glucose to the anthocyanidin. The addition of glucose or other sugar molecules can alter anthocyanins' chemical and physical properties, including their color, solubility, and stability.

Figure 2. Cyanidin (anthocyanidin, **left**) and its 3-*O*-glycosyl product chrysanthemin (anthocyanin, **right**) derived from the enzymatic activity of glucosyltransferase.

I-1.2 Diversity of anthocyanins in nature

Among the most frequently encountered monomeric anthocyanins are the glycosides of cyanidin, delphinidin, malvidin, and pelargonidin **(Figure 3)**. These anthocyanins are widely distributed in plants and contribute to the red, blue, and purple colors of flowers, fruits, and other plant tissues. The color of these compounds is closely tied to their ability to absorb light at specific wavelengths, which is determined by their molecular structure and the arrangement of conjugated bonds in the flavonoid backbone. The colors of anthocyanins can shift depending on their concentration, the surrounding environmental factors, and the presence of other pigments, such as chlorophyll, which can alter the overall appearance of plant tissues.

Anthocyanins are not only responsible for the visual appeal of plants but also play essential protective roles. In some plants, their accumulation is a defensive mechanism to protect the plant from herbivory. The bright colors of flowers and fruits, which are often rich in anthocyanins, help attract pollinators and seed dispersers. However, the accumulation of anthocyanins can also serve to reduce the plant's strong green coloration, which could make it more susceptible to herbivorous predators. This modification of color serves as a form of

camouflage or deterrence, reducing the plant's visibility to potential herbivores, thereby increasing its chances of survival.

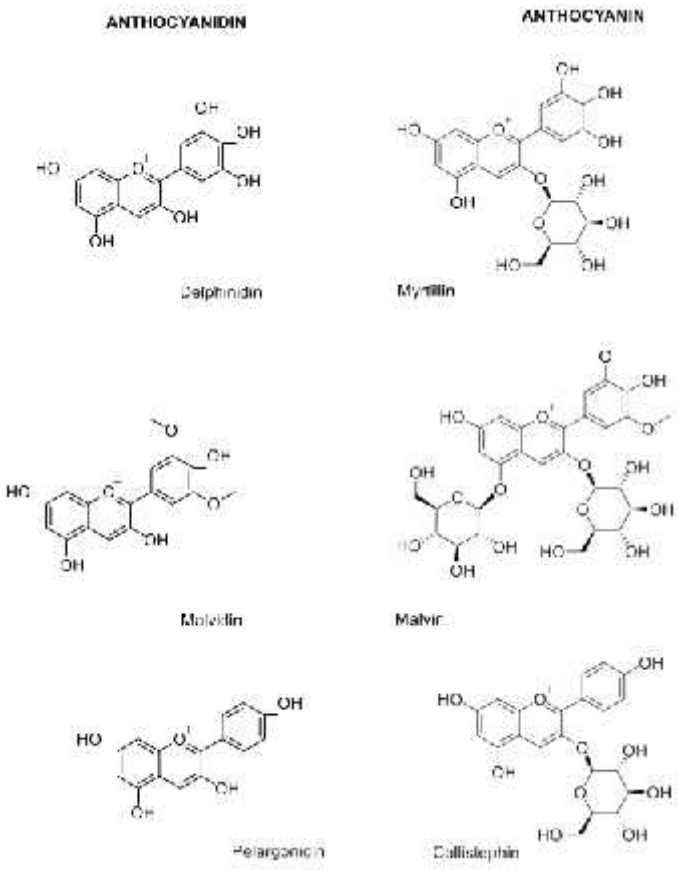

Figure 3. Chemical structure of some naturally occurring anthocyanidins (**left**) and corresponding mono- or di-glycosylated anthocyanins (**right)**

I-1.3 pH-Dependent coloration and stability of anthocyanins

The ability of anthocyanins to produce different colors is closely related to their pH-dependent ionization states. At acidic pH, anthocyanins predominantly exist as flavylium cations (positively charged), which impart red colors to plant tissues. As the pH increases toward neutral conditions, the anthocyanins lose their charge and are converted into uncharged quinone forms, which appear less colorful. This change in color with pH is a direct result of the electronic conjugation properties around the oxonium moiety of the anthocyanin molecule, which alters its absorbance characteristics.

At basic pH, anthocyanins are less stable and tend to degrade into dark brown, oxidized compounds. The degradation of anthocyanins at alkaline pH is primarily due to the loss of their conjugated electronic structure and the breakdown of their chemical bonds, leading to the loss of their vibrant color. This pH-dependent behavior is crucial in the natural function of anthocyanins in plants, where color change may serve both protective and communicative purposes, and in the food industry, where the stability of anthocyanins in food products is essential for maintaining color quality **(Figure 4)**.

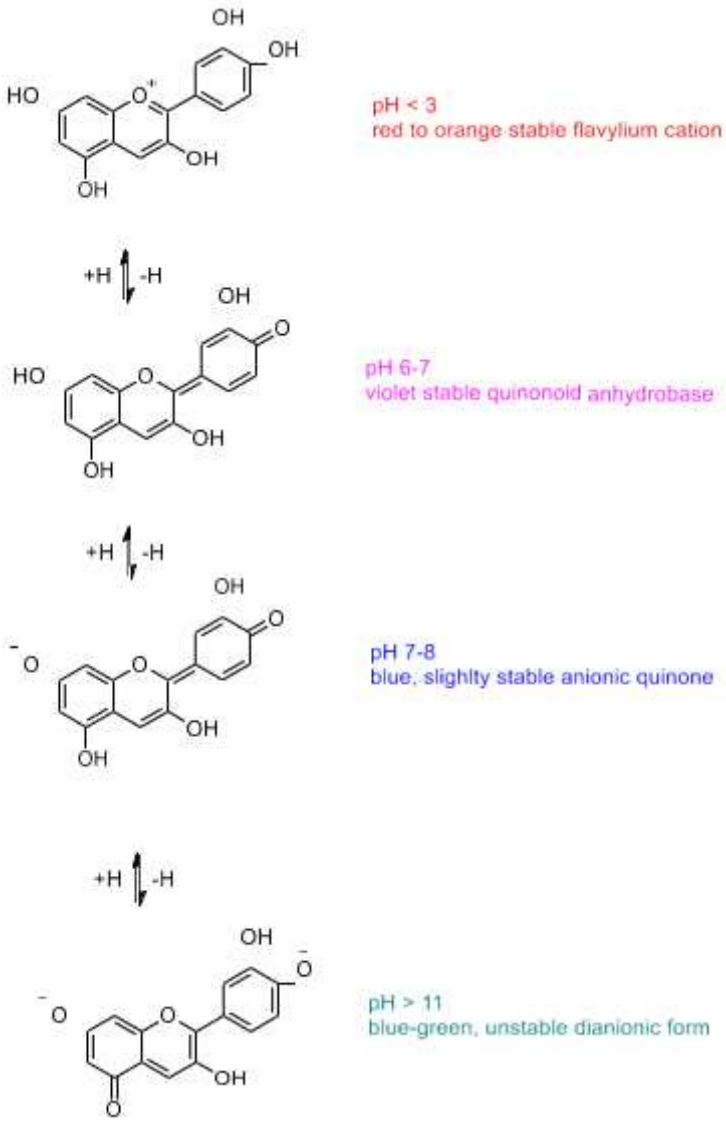

Figure 4. Molecular states and electronic delocalization of cyaniding at different pH values

I-1.4 Color shifts and defense mechanisms in plants

The pH sensitivity of anthocyanins, along with their capacity to interact with other plant pigments such as chlorophyll, is an important factor in the coloration of plant tissues. In many plants, especially flowers and fruits, the combined light absorbance of both chlorophyll and anthocyanins contributes to the overall color perceived by humans and animals. This dual role of anthocyanins—attracting pollinators and protecting plants from environmental stress—demonstrates the complexity of plant pigment functions. The shift from a green (chlorophyll-dominated) to a red or purple (anthocyanin-dominated) color under certain environmental conditions is visually striking and serves as an adaptive mechanism for plant survival.

The stability of anthocyanins and their ability to provide color in different environments depend on various factors, including temperature, light exposure, and the presence of metal ions. The electronic properties of the anthocyanin molecule, which are influenced by its structure and the surrounding conditions, are responsible for the absorption of light at specific wavelengths and the resultant color display. As such, the biochemistry of anthocyanins is critical for understanding their role in plant physiology and for harnessing their potential applications in food science, nutrition, and health.

I-2 Extraction, purification, and chemical characterization

A wide range of strategies have been developed for extracting anthocyanins and anthocyanidins from biological matrices. The choice of strategy depends on the complexity of the matrix and the specific chemical properties of the target molecules **(Table I).** Extraction procedures differ according to whether the objective is to recover total anthocyanidins or individual anthocyanins, as these compounds exhibit distinct solubility profiles and chemical characteristics.

Table I . Overview of Methods for Anthocyanin and Anthocyanidin Analysis

Aspect	Methods	Key Features
Extraction	Solvent systems (Ethanol, Methanol, NADES)	Acidification for stability; tailored to the polarity of the target compound.
Purification	SPE, Chromatography (Reverse-phase, HSCCC)	Selective sorbents (C18, Sephadex); biphasic systems for high-resolution separation.
Characterization	MS, FT-IR, NMR	Mass fragmentation pathways; vibrational spectra for functional groups; detailed atom-level structural insights.

I-2.1 Extraction of anthocyanins

I-2.1.1 Solvent Selection

Aqueous/organic solvent mixtures containing a higher proportion of organic solvent are typically employed to extract total anthocyanidins. In contrast, anthocyanins are preferentially extracted using more hydrophilic solvents or aqueous-based mixtures. In all cases, preserving the ionization state of the compounds in their flavylium form is essential to maintain stability and antioxidant properties. This is achieved by acidifying the aqueous phase with inorganic or organic acids.

The most commonly used systems consist of ethanol or methanol/water mixtures (70–95:30–5, v/v) acidified with hydrochloric acid (HCl), formic acid, or citric acid, and are widely applied to plant, food, and agricultural samples. Aqueous acetone (70%) is another solvent system used for specific extraction purposes. For matrices resistant to acidic homogenization, such as plant seeds, stronger and more lipophilic organic solvents combined with auxiliary techniques—including ultrasonication and elevated temperatures—may be required to facilitate extraction.

Liquid matrices such as red wine, pomace, and fruit juices are generally treated with acidic alcoholic solutions to isolate anthocyanins. More recently, natural deep eutectic solvents (NADES) have attracted growing interest as biocompatible and eco-friendly alternatives to conventional organic solvents. NADES have been reported to provide higher extraction yields

compared to exhaustive extractions with traditional solvents, representing a promising green extraction strategy, particularly for pharmaceutical and nutraceutical applications.

I-2.1.2 Minimizing Degradation

Maintaining the structural integrity of anthocyanins throughout the extraction process requires careful control of pH, temperature, light exposure, and oxygen levels. As described above, acidification of the extraction medium is critical to stabilize anthocyanins in their colored flavylium cation form, which is the most stable ionic species at low pH. Avoiding alkaline conditions and prolonged heat exposure is equally important, as these factors promote hydrolysis, ring-opening reactions, and irreversible degradation of the pigments.

The use of antioxidants such as ascorbic acid, and the exclusion of light and oxygen during extraction and storage, can significantly reduce oxidative degradation. When elevated temperatures are necessary to improve extraction efficiency—particularly from rigid or lipophilic matrices—processing times should be minimized and temperatures carefully controlled to limit thermal degradation. Collectively, these precautions ensure the recovery of structurally intact anthocyanins suitable for subsequent purification and analysis.

I-2.2 Purification and fractionation

Anthocyanin purification involves separating the target pigments from co-extracted compounds such as sugars, organic acids, proteins, and other polyphenols. The appropriate purification strategy depends on the composition of the crude extract and the desired degree of purity.

For aqueous extracts, solid-phase extraction (SPE) using C18 cartridges or Sephadex LH-20 matrices is commonly employed to selectively retain anthocyanins while allowing more polar, non-retained by-products to pass through. Further chromatographic purification may involve normal-phase silica gel, reverse-phase, or cation exchange chromatography. Ion exchange resins such as Amberlite IRC 80, Amberlite XAD-7HP, and DOWEX 50WX8 allow for additional refinement of the anthocyanin fraction.

When the extract contains significant chlorophyll content, an initial step to remove green pigments is required before proceeding with anthocyanin purification. Extracts already rich in anthocyanins—such as those derived from fruits, flowers, or liquid biological samples—can typically proceed directly to chromatographic purification.

High-speed counter-current chromatography (HSCCC) is a particularly effective technique for isolating pure anthocyanin compounds following an initial enrichment using XAD-7 resin columns. HSCCC exploits liquid–liquid partitioning using biphasic solvent systems, such as n-butanol/ethyl acetate/0.5% acetic acid (3:1:4, v/v/v) or 0.2% trifluoroacetic acid/n-butanol/tert-butyl methyl ether/acetonitrile (6:5:2:1, v/v/v/v), both of which have demonstrated excellent chromatographic performance. The purity and identity of isolated compounds are subsequently confirmed by HPLC and NMR analysis.

Additional conventional techniques employed at various stages of the purification workflow include thin-layer chromatography (TLC), used as a simple and cost-effective tool for monitoring separation, and column chromatography (CC) with a range of stationary phases such as silica gel, reversed-phase silica gel, polyamides, and polymeric resins.

Once pure compounds are obtained through serial or parallel chromatographic separations, they can be subjected to detailed structural characterization using advanced spectroscopic techniques, including nuclear magnetic resonance (NMR), high-resolution and tandem mass spectrometry (HR-MS/MS^n), and Fourier-transform infrared spectroscopy (FT-IR).

I-2.3 Analytical methods and chemical characterization

The analysis of anthocyanins requires precise and sensitive techniques capable of identifying, quantifying, and structurally characterizing these compounds in complex biological matrices. The principal analytical approaches include spectrophotometric measurements, various chromatographic techniques, and advanced spectroscopic methods such as mass spectrometry, NMR, and infrared spectroscopy.

I-2.3.1 Spectrophotometric measurements

Spectrophotometric methods are widely used for the quantification of total monomeric anthocyanins in plant extracts, food products, and agricultural samples. These methods exploit the characteristic light absorption properties of anthocyanins, which are directly related to their ionization state. In acidic environments, anthocyanins exhibit a distinctive red-orange color associated with a high molar extinction coefficient in the visible region, typically between 500 and 550 nm. The precise absorption maximum and extinction coefficient vary depending on the specific anthocyanin structure.

Figure 4 illustrates the different electronic delocalization states of anthocyanins across various pH ranges, each corresponding to a distinct color. This chromatic variation reflects the extent of electronic conjugation between rings A and B through ring C. Anthocyanins display their most intense coloration at low pH values, where the conjugation system is fully developed across the flavylium cation. As pH increases, this conjugation is progressively disrupted, resulting in spectral shifts and color changes. At very high pH, anthocyanins undergo degradation and lose their color entirely. A colorless pH range also exists in which anthocyanins retain their structural integrity but do not exhibit visible light absorption.

Between pH 4 and 5, anthocyanins adopt the hemiketal (carbinol pseudobase) form, in which electronic delocalization among the three aromatic rings is disrupted, resulting in the absence of color. This form is commonly encountered during purification and quantification procedures.

Figure 5 depicts the stable colored and colorless forms of cyanidin at different pH values, illustrating the structural and chromatic transformations associated with pH variation.

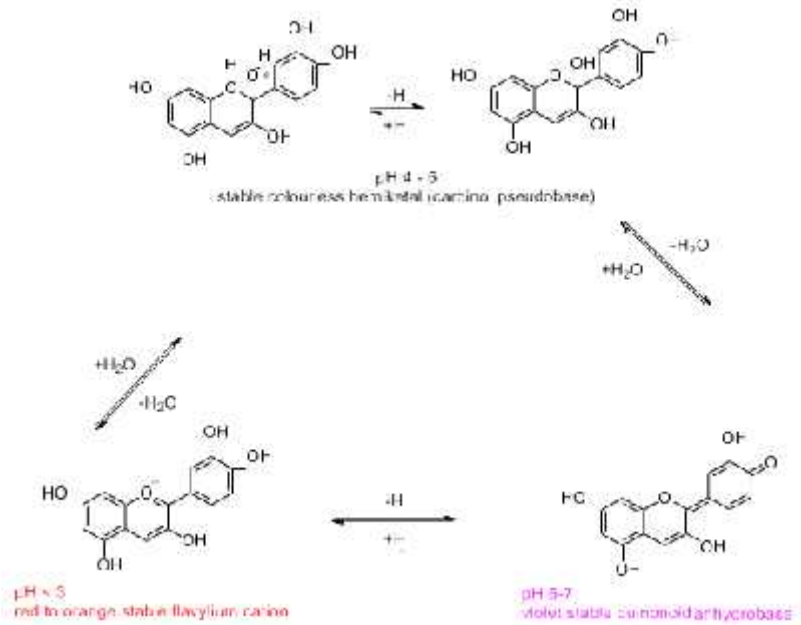

Figure 5. Stable colored and colorless forms of cyanidin at different pH values.

The pH-differential method is the most reliable spectrophotometric approach for quantifying monomeric anthocyanins. It involves recording UV-visible absorption spectra at two pH values, typically pH 1.0 and pH 4.5.

Figure 6 shows the absorption spectra of cyanidin-3-O-β-glucoside at both pH conditions. The difference in absorbance between the two pH values in the visible range provides an accurate estimate of total monomeric anthocyanin concentration, even in the presence of other colored pigments or interfering substances.

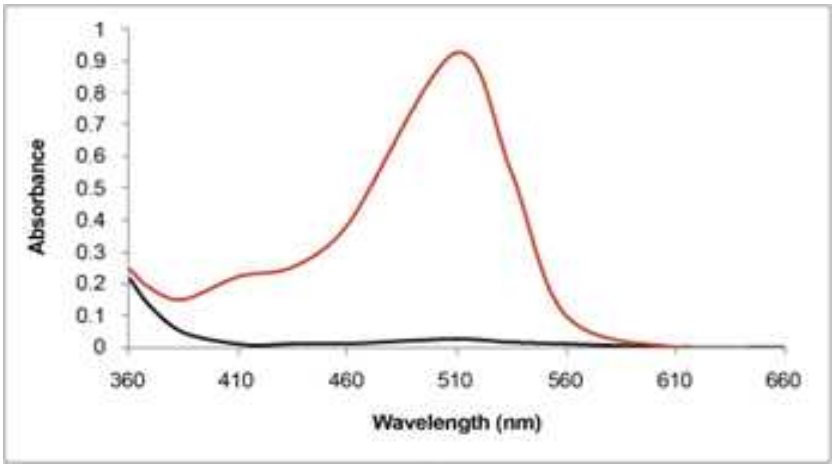

Figure 6. UV-visible absorption spectra of cyanidin-3-*O*-β-glucoside at pH 1.0 (**red line**) and pH 4.5 (**black line**).

The general formula used to calculate anthocyanin concentration by this method is presented below:

$$\Delta Abs = (\mathrm{Abs}_{\lambda_{max}} \text{ at pH } 1.0) - (\mathrm{Abs}_{\lambda_{max}} \text{ at pH } 4.5)$$

To quantify anthocyanins in complex biological extracts, the concentration of the predominant anthocyanin is calculated using the differential absorbance value (ΔAbs), together with the molecular weight (MW) and molar extinction coefficient (ε) of the relevant anthocyanin:

$$\text{Anthocyanin concentration (mg/mL)} = \frac{(\Delta Abs \times MW \times \text{dilution factor})}{\varepsilon}$$

For unknown or incompletely characterized samples, cyanidin-3-O-β-glucoside (chrysanthemin) is used as a reference standard. Its MW (449.2 g/mol) and molar extinction coefficient (ε = 25,740 $L \cdot mol^{-1} \cdot cm^{-1}$; 0.1 N aqueous HCl, λmax 520 nm) are applied to calculate total anthocyanin content.

An additional important step in spectrophotometric analysis is the estimation of degraded and polymerized anthocyanins, which may contribute to the overall color intensity of a sample. The bisulfite bleaching method is commonly employed for this purpose. Monomeric anthocyanins react with bisulfite at the electrophilic C-4 position of the flavylium ring, forming sulfonic acid adducts that lack the oxonium moiety responsible for coloration, thereby halting electronic conjugation and rendering the solution colorless.

Figure 7 illustrates the transformation of the flavylium cation (left) into the colorless cyanidin–sulfonic acid adduct (right) upon reaction with bisulfite. This reaction is exploited to assess the contribution of polymeric anthocyanin species to the total color intensity of a sample.

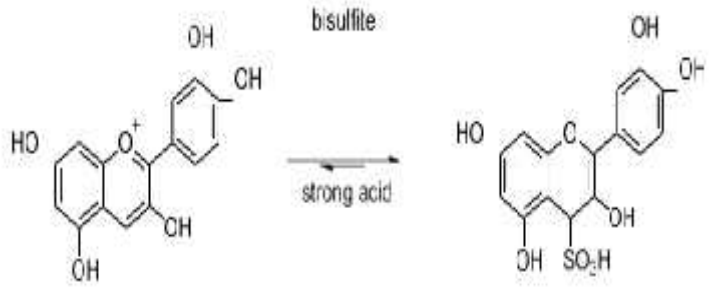

Figure 7. Flavylium cation form (**left**) and colorless cyanidin–sulfonic acid adduct after bleaching reaction with bisulfite (**right**).

The bisulfite method involves measuring absorbance before and after bisulfite treatment. The treated sample reflects the color contributed solely by polymeric species, which are resistant to bisulfite bleaching, while the untreated sample represents total color density from both monomeric and polymeric forms. Comparing the absorbance values of both samples

enables estimation of the relative contribution of polymerized anthocyanins. This approach is particularly valuable for assessing the stability and degree of anthocyanin polymerization in complex biological samples, providing insights into structural modifications that may occur during extraction, storage, or processing.

I-2.3.2 Chromatographic Analyses

Chromatographic analyses of anthocyanins are primarily based on high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC), typically coupled with UV-visible spectrophotometric and/or mass spectrometric detection. These techniques are widely used for separating and identifying anthocyanins in complex biological matrices due to their high resolution and sensitivity.

Reverse-phase columns with C18 silica-bonded stationary phases are the most commonly employed for flavonoid analysis, including anthocyanins. Column particle sizes of 5 μm remain standard in many analytical laboratories; however, columns with smaller particle sizes (2.6 μm or 1.7 μm) are increasingly used with modern UHPLC instruments to improve resolution and throughput. Both HPLC and UHPLC systems provide satisfactory separation of anthocyanins, even in complex sample matrices.

Reverse-phase methods typically employ aqueous/organic gradients composed of acidified water and organic solvents such as acetonitrile or methanol. The inclusion of formic acid in the mobile phase is particularly advantageous for mass spectrometric coupling, as it enhances the ionization efficiency of anthocyanins. The low flow rates characteristic of UHPLC systems are also well suited for interfacing with mass spectrometers, including single and triple quadrupole instruments, enabling highly sensitive detection and quantification.

HPLC systems equipped with photodiode array detectors (DAD) are the most widely used configuration for the determination and quantification of anthocyanins. By monitoring specific wavelengths (e.g., 520 nm) and applying linear calibration curves constructed from high-purity analytical standards, these methods permit precise and reliable quantification, often enabling simultaneous analysis of multiple compounds within a single analytical run. Numerous validated HPLC methods are documented in the literature, with variations in column selection and gradient conditions designed to optimize the separation of specific anthocyanins.

Although a substantial number of anthocyanins are commercially available as pure analytical standards, many—particularly those bearing unusual sugar moieties or representing

recently discovered structures—lack commercially available standards. In such cases, UHPLC or HPLC coupled with mass spectrometry becomes indispensable. Anthocyanins are naturally occurring charged compounds that are stabilized in their oxonium form under acidic conditions, rendering them highly compatible with the acidic mobile phases standard in reversed-phase HPLC/UHPLC systems.

A key advantage of mass spectrometric detection is its capacity to monitor individual molecular ions through single ion recognition (SIR) or single ion monitoring (SIM) techniques, enabling the selective detection and quantification of co-eluting compounds that would otherwise be unresolvable. Furthermore, mass spectrometry provides essential structural information for identifying unknown compounds in the absence of commercial reference standards, by integrating chromatographic retention times, UV-visible spectral data, and accurate molecular masses.

For comprehensive structural characterization, tandem mass spectrometry (MS/MS^n) coupled with HPLC or UHPLC is an essential tool. MS/MS^n enables the examination of multiple fragmentation transitions, which can be compared against literature data or mass spectrometric databases. This multi-stage approach delivers detailed structural insight into anthocyanin identity, facilitating their identification and quantification with high precision, even in chemically complex biological matrices.

Mass spectrometry is particularly informative for differentiating anthocyanins from their aglycones (anthocyanidins): the characteristic loss of sugar moieties following MS/MS fragmentation distinguishes glycosylated from non-glycosylated forms, while cross-ring cleavages—especially on ring C—give rise to diagnostic oxonium fragment ions specific to the aglycone.

FT-IR spectroscopy provides complementary information on functional group composition. Characteristic absorption bands include skeletal stretching vibrations of aromatic rings and C–O–C groups in flavonoids (approximately 1072, 1261, and 1516 cm^{-1}), C–O stretching vibrations (around 1711 cm^{-1}), and carbohydrate-associated bands (near 1072 cm^{-1}), the latter allowing discrimination between anthocyanins and anthocyanidins.

While MS/MS and FT-IR represent powerful tools for the identification of known anthocyanins and anthocyanidins, NMR spectroscopy remains the gold standard for unequivocal structural elucidation of newly isolated compounds. A diagnostic feature in the proton (^1H) NMR spectra of anthocyanins is the characteristic low-field singlet at 8.6–9.1 ppm,

attributable to the H-4 proton of the flavylium salt. Aliphatic sugar protons appearing in the high-field region (3.5–5.5 ppm) distinguish anthocyanins from their aglycones. Anomeric proton and carbon signals are shifted downfield relative to other sugar resonances, enabling identification of glycosidic linkages, while the magnitude of anomeric coupling constants indicates the α or β configuration of the sugar residue.

Two-dimensional NMR techniques—including homonuclear ($^{1}H–^{1}H$) and heteronuclear ($^{1}H–^{13}C$) experiments such as TOCSY, HSQC, HMBC, and ROESY/NOESY—provide detailed information on atomic connectivity and stereochemistry for both the aglycone and sugar moieties. These methods are indispensable for the comprehensive characterization of complex anthocyanins and novel anthocyanin structures identified in botanical sources.

II- Modification and stabilization of anthocyanins

Anthocyanins are highly susceptible to degradation under various conditions, including changes in pH, temperature, light exposure, and the presence of oxygen, enzymes, and metal ions. This instability leads to color loss, reduced bioactivity, and diminished therapeutic potential during processing, storage, and digestion. Consequently, extensive research has focused on developing strategies to modify and stabilize anthocyanins, thereby enhancing their shelf life, bioavailability, and functional properties. Various approaches have been explored, including chemical modifications such as acylation and glycosylation, physical methods like encapsulation and complexation with metals or proteins, and enzymatic transformations. These stabilization techniques aim to protect anthocyanins from environmental stressors while maintaining or even improving their biological activities, ultimately enabling their broader application in the food, nutraceutical, and pharmaceutical industries.

II-1 Glycosyl transferases

II-1.1 Glycosylation of anthocyanins

Anthocyanins, a subclass of flavonoids, are natural pigments responsible for the vivid red, purple, and blue colors observed in many fruits, vegetables, and flowers. They exist predominantly in their glycosylated forms, where one or more sugar moieties are covalently bonded to the anthocyanidin aglycone. This glycosylation plays a crucial role in defining the physicochemical properties, stability, and biological functions of anthocyanins.

II-1.1.1 Structure and glycosylation patterns of anthocyanins

The core structure of anthocyanins consists of an anthocyanidin (aglycone), characterized by a C6-C3-C6 carbon skeleton with hydroxyl and/or methoxyl substitutions. Glycosylation typically occurs through the substitution of hydroxyl groups on specific positions of the anthocyanidin backbone. These positions include (**Table II)** :

- C-3 (most common)
- C-5, C-7 (on the A-ring)
- C-3', C-4', and C-5' (on the B-ring)

Atypical glycosylation has been observed in some species. For instance, C-glycosylation at the C-8 position is a rare modification noted in *Tricyrtis formosana*. The sugar moieties commonly involved in glycosylation include glucose, galactose, xylose, glucuronic acid, and

arabinose. These sugar residues can be further modified by additional glycosylation or acylation, enhancing the functional versatility of anthocyanins.

Table II. Common glycosylation sites and sugar moieties in anthocyanins

Glycosylation Site	Sugar Moiety	Example Compound	Functional Implication
C-3	Glucose	Cyanidin-3-O-glucoside	Enhanced water solubility and color stability
C-5	Glucose, Galactose	Delphinidin-5-O-galactoside	Improved antioxidant activity
C-7	Glucuronic acid	Peonidin-7-O-glucuronide	Increased metabolic stability
C-8	Xylose (rare)	Specific to *Tricyrtis formosana*	Rare structural feature
C-3', C-4', C-5'	Arabinose	Malvidin derivatives	Stabilized pigmentation under acidic conditions

Certain anthocyanidins, such as 3-desoxyanthocyanidins and pyranoanthocyanidins, can exist in both glycosylated and unglycosylated forms, which is uncommon among flavonoids. These unique forms provide specific functional and structural diversity within the anthocyanin family.

II-1.1.2 Role of glycosylation in anthocyanin stability

Glycosylation has significant implications for the stability and functionality of anthocyanins:

1. **Increased hydrophilicity:** The addition of sugar moieties enhances the solubility of anthocyanins in aqueous environments, overcoming the hydrophobic nature of the anthocyanidin aglycone. This increased hydrophilicity facilitates their incorporation into plant vacuoles and their role as pigments in plant tissues.

2. **Physiological stability:** Anthocyanidins, in their aglycone form, are inherently unstable under physiological pH and oxidative conditions. Glycosylation mitigates this instability, allowing anthocyanins to accumulate in plant tissues effectively. For instance, plants deficient in anthocyanidin 3-O-glucosylation exhibit reduced anthocyanin accumulation due to the instability of non-glycosylated anthocyanidins.

3. **Color stability:** The vibrant colors of anthocyanins are heavily influenced by their chemical structure. Glycosylated anthocyanidins display color instability in mildly acidic aqueous solutions unless further stabilized by aromatic acylation. The aromatic acyl groups, often linked to the glycosyl residues, provide additional stability to the chromophore through intramolecular copigmentation.

4. **Signal for vacuolar transport:** Glycosylation serves as a signal for the intracellular transport of anthocyanins to the vacuole. This compartmentalization is essential for their role in pigmentation and other physiological functions. Vacuolar sequestration also prevents the potential cytotoxic effects of free anthocyanidins.

II-1.1.3 Mechanisms of glycosylation and functional diversification

Anthocyanin glycosylation is mediated by glycosyltransferase enzymes that catalyze the transfer of sugar residues to specific hydroxyl groups on the anthocyanidin structure. The degree and type of glycosylation can vary across plant species, leading to the remarkable diversity observed in anthocyanin profiles. This diversity is vital for plant adaptation and survival, influencing attributes such as UV protection, pollinator attraction, and pathogen resistance.

II-1.1.4 Applications and implications in food science and technology

Anthocyanins are widely recognized for their health benefits, including antioxidant, anti-inflammatory, and cardioprotective properties. Glycosylation significantly impacts their functional properties in food systems:

- **Colorant stability**: Glycosylated anthocyanins are preferred for use as natural food colorants due to their enhanced stability in various pH ranges.
- **Bioavailability**: Glycosylation influences the absorption and metabolism of anthocyanins in the human gastrointestinal tract.

II-1.2 Anthocyanin glycosyl transferases

II-1.2.1 Role of glycosyltransferases (UGTs) in flavonoid metabolism

Anthocyanin O-glycosylation is a pivotal biochemical modification catalyzed by family 1 glycosyltransferases (UGTs) (**Table III)**. These enzymes utilize flavonoids, including anthocyanidins, as sugar acceptors and a range of UDP-sugars as sugar donors. The process involves the transfer of sugar moieties to specific hydroxyl groups on the flavonoid aglycones,

thereby enhancing the solubility, stability, and functionality of these compounds within plant tissues.

Table III. Common glycosyl transferases involved in anthocyanin glycosylation

Enzyme	Catalytic Activity	Preferred Substrates	Phylogenetic Cluster
A3GlcT	C-3 O-glucosylation	Anthocyanidins, flavonols	I
A3GalT	C-3 O-galactosylation	Anthocyanidins, flavonols	I
A5GlcT	C-5 O-glucosylation	Anthocyanidins	II
A5,3GlcT	Sequential C-5 and C-3 glucosylation	Anthocyanidins	III
A3G-2ᅟᅟGlcT	C-3 O-glucoside glucosylation	Anthocyanidin 3-O-glucosides	IV
Citrus Flavanone 7-RhaT	C-7 O-neohesperidoside formation	Flavanone 7-O-glucosides	IV

- ***Mechanism of UGT Activity***

UGTs demonstrate regioselectivity in glycosylation, targeting specific hydroxyl groups on the flavonoid backbone. The primary sugar donors include UDP-glucose, UDP-galactose, UDP-rhamnose, UDP-xylose, UDP-glucuronic acid, and UDP-arabinose. The enzyme-substrate specificity is governed by the molecular architecture of the UGTs, particularly the presence of a conserved Plant Secondary Product Glycosyltransferase (PSPG) motif. This motif facilitates the binding of nucleotide-diphosphate sugars, playing a critical role in the catalytic process.

- ***Evolutionary Insights and Functional Clusters***

A phylogenetic analysis of UGT amino acid sequences reveals distinct evolutionary clusters associated with regioselective glycosylation. For example, enzymes responsible for C-3 glucosylation (A3GlcT) cluster separately from those catalyzing C-5 glucosylation (A5GlcT). Interestingly, some enzymes, such as the rose anthocyanin 5,3-O-glucosyltransferase (A5,3GlcT), display unique catalytic pathways, glucosylating at the C-5 position prior to C-3, and forming intermediates that rapidly convert to diglucoside forms without detectable intermediates.

- ***Substrate Specificity and Functional Adaptation***

Enzymes like A3GlcT and A3GalT exhibit broad substrate specificity, accepting both anthocyanidins and flavonols. Conversely, enzymes such as petunia flavonol 3-GalT (F3GalT) demonstrate functional specialization, catalyzing reactions primarily in pollen and suggesting an adaptive evolutionary trajectory for reproductive success.

In most plant species, glycosylation at the C-3 hydroxyl group is the primary modification step. This modification is essential for enhancing the hydrophilicity and color stability of anthocyanins, critical traits for attracting pollinators and providing UV protection. However, exceptions, such as gentian A3£GlcT, belong to subfamilies (e.g., flavonoid 7UGT) that extend beyond anthocyanin metabolism.

II-1.2.2 Role of glycosylation in anthocyanin functionality

Glycosylation significantly influences anthocyanin bioavailability, stability, and functional properties. Key aspects include:

- **Stability enhancement**: Glycosylation protects anthocyanins from degradation under physiological and environmental conditions, including acidic pH and oxidative stress.
- **Hydrophilicity**: The addition of sugar moieties increases water solubility, facilitating vacuolar transport and storage.
- **Color intensity and stability**: The glycosylation of anthocyanins, coupled with acylation, stabilizes their color in plant tissues, critical for plant signaling and reproduction.

II-2 Methyltransferases

Methylation is a critical post-synthetic modification of flavonoids, a diverse group of polyphenolic compounds widely distributed in the plant kingdom. This process impacts flavonoid solubility, stability, and bioactivity, playing a significant role in plant physiology and human health applications.

II-2.1 Anthocyanidins and their methylated derivatives

Anthocyanins are glycosylated derivatives of anthocyanidins and constitute one of the major flavonoid subclasses responsible for the vivid pigmentation in flowers, fruits, and leaves. Among the six primary anthocyanidins—pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin—methylation is predominantly observed in peonidin, petunidin, and malvidin. These three methylated forms account for approximately 20% of all reported anthocyanins.

II-2.2 Enzymatic methylation by O-methyltransferases (OMTs)

O-Methyltransferases (OMTs) are enzymes responsible for catalyzing the methylation of flavonoids and other secondary metabolites. Their activity is dependent on S-adenosyl-L-

methionine (SAM), which serves as the methyl donor. OMTs are categorized into two classes based on their molecular weight and dependence on divalent cations like Mg^{2+}:

1. **Class I OMTs**
    - Molecular weight: 23–27 kDa
    - Mg^{2+}-dependent
    - Conserved motifs (A, B, and C) associated with SAM binding and activity.
    - Examples: Caffeoyl CoA 3-OMTs
2. **Class II OMTs**
    - Molecular weight: 38–43 kDa
    - Mg^{2+}-independent
    - Similar conserved motifs with slight differences in spacing.
    - Examples: Flavonoid OMTs (e.g., flavonol 3′-OMT, flavonoid 7-OMT), Isoflavone OMT, Caffeic acid 3-OMTs and Catechol OMTs

II-2.3 Anthocyanin-Specific OMTs (AOMTs)

The anthocyanin biosynthetic pathway, extensively studied in model plants like *Petunia hybrida*, *Antirrhinum majus* (snapdragon), and maize, highlights the specialization of anthocyanin-specific OMTs (AOMTs). These enzymes, predominantly localized in the cytosol, catalyze the methylation of anthocyanins, influencing pigment diversity and stability.

a) Gene Encoding AOMTs

- *Mt1/Mt2*: Encode anthocyanin 3′-OMTs
- *Mf1/Mf2*: Encode anthocyanin 3′,5′-OMTs

The expression and activity of these genes are strongly correlated with specific genotypes, such as *An1* and *An2* in petunia.

b) Enzyme Localization and Functionality

AOMTs are predominantly class I OMTs, contrasting with flavonoid OMTs, which are class II. For instance, AOMTs from petunia and *Torenia hybrida* catalyze the methylation of delphinidin derivatives, such as delphinidin 3-O-glucoside.

II-3 Acyl transferases

II-3.1 Acylation of anthocyanins

Acylation represents a pivotal biochemical modification of plant secondary metabolites, prominently including anthocyanins. These water-soluble pigments are critical to plant coloration, serving ecological roles such as pollinator attraction and stress resistance. Acylation diversifies anthocyanin structures by the addition of aromatic or aliphatic acyl substituents, typically attached to glycosyl moieties. This modification (**Table IV)** enhances the functional versatility of anthocyanins by influencing their solubility, stability, and chromatic properties.

Table IV. Types of acyl substituents and their effects on anthocyanins

Type of Acyl Substituent	Examples	Attachment Site	Effects
Aromatic Acyl Groups	– Hydroxycinnamoyl (e.g., p-coumaryl, caffeoyl, feruloyl, sinapoyl) – Gallyl groups	6-position of glycosyl residues	– Increased structural diversity – Enhanced color stability – Bluing effect (via intramolecular stacking)
Aliphatic Acyl Groups	– Malonyl – Acetyl – Succinyl – Malyl – Oxalyl – Tartaryl	6-position of glycosyl residues	– Improved aqueous solubility – Enhanced stability of coloration (e.g., malonylation)
Polyacylation	Multiple aromatic acyl groups (e.g., hydroxycinnamoyl groups at 7- and 3'-positions)	Multiple positions on anthocyanins	– Highly stable coloration – Intense and vibrant blue hues (e.g., in gentian, butterfly pea)
Dicarboxylic Acyl Groups	– Malonyl – Malyl	Glycosyl residues	– Buffering of vacuolar pH – Enhanced solubility in aqueous environments
Non-Acylated Anthocyanins	None	Not applicable	– Rapid decoloration in neutral or weakly acidic conditions – Hydration at C-2 position of flavylium nucleus leading to instability

II-3.1.1 Structural diversity induced by acylation

Over 65% of characterized anthocyanins are acylated, demonstrating the prevalence and importance of this modification. Aromatic acyl substituents generally involve hydroxycinnamoyl groups (e.g., p-coumaryl, caffeoyl, feruloyl, sinapoyl), which contribute

significantly to structural complexity. Notable exceptions include gallyl groups. Aliphatic acyl substituents encompass malonyl, acetyl, succinyl, malyl, oxalyl, and tartaryl groups, with malonyl being the most common. These acyl groups are often linked to the 6-position of glucosyl residues, as observed in anthocyanins derived from carrot (*Daucus carota*) and parsley (*Petroselinum hortense*).

Acylation facilitates the selective transport of anthocyanins into vacuoles, a mechanism essential for compartmentalization and storage within plant cells. This process highlights the functional interplay between biochemical modifications and cellular physiology.

II-3.1.2 Impact on stability and coloration

Anthocyanins lacking acyl groups exhibit reduced stability in neutral or weakly acidic aqueous solutions due to hydration at the C-2 position of the anthocyanidin flavylium nucleus. In contrast, acylation stabilizes anthocyanin coloration, particularly under similar conditions.

Polyacylation (the attachment of multiple aromatic acyl groups) is particularly effective, enhancing both color stability and intensity. This phenomenon underpins the vibrant and enduring blue hues in flowers such as gentian (*Gentiana triflora*), cineraria (*Senecio cruentus*), and butterfly pea (*Clitoria ternatea*). The stabilization effect arises from intramolecular face-to-face stacking interactions between aromatic acyl groups and the anthocyanidin nucleus. This interaction minimizes chromophore destabilization and extends color retention.

The stability of anthocyanin coloration depends on the number and positions of aromatic acyl groups. For instance, aromatic acylation at both the 7- and 3'-positions of glycosyl groups produces the most stable blue flower colors. Conversely, aliphatic acylation, though not altering the absorption spectra, contributes to enhanced stability. Cyanidin derivatives illustrate this effect, with malonylation significantly increasing coloration stability at pH 5 and 7 compared to their non-acylated counterparts.

II-3.1.3 Biochemical and functional significance

Acylation improves anthocyanin solubility by introducing dicarboxylic acids such as malonyl and malyl groups, which enhance aqueous solubility and facilitate vacuolar buffering. These acyl groups maintain vacuolar acidity, stabilizing coloration and reinforcing the visual appeal of flowers.

Furthermore, acylation confers resistance to enzymatic degradation. Most microbial glycosidases are incapable of hydrolyzing acylated glycosides, providing biochemical stability and safeguarding anthocyanins against indiscriminate degradation. This protective mechanism extends the functional longevity of stored anthocyanins within plant cells.

II-3.2 Anthocyanin acyl transferases

The process of acylation in anthocyanins is facilitated by anthocyanin acyltransferases (AATs), a group of specialized enzymes that catalyze the transfer of acyl groups to anthocyanins. This enzymatic activity is critical for the structural diversification and functional enhancement of anthocyanins, influencing their solubility, stability, and coloration properties.

II-3.2.1 Types of anthocyanin acyltransferases (AATs)

a. BAHD Superfamily of AATs

- **Acyl Donors**: These enzymes use acyl coenzyme A thioesters (acyl-CoA) as their acyl donors.
- **Mechanism**: The BAHD enzymes catalyze the transfer of the acyl group from acyl-CoA to the hydroxyl groups of the anthocyanin molecules.
- **Distribution**: Acyl-CoA-dependent AATs have been widely identified across various plant species.
- **Molecular Characterization**: These enzymes have been extensively studied and characterized at the molecular level, providing insights into their catalytic mechanisms and structural properties.

b. Serine Carboxypeptidase-Like (SCPL) Group of AATs

- **Acyl Donors**: SCPL enzymes utilize acyl-activated sugars, such as β-acetal esters or 1-O-β-glucose esters, as acyl donors.
- **Mechanism**: Unlike the BAHD family, SCPL enzymes transfer the acyl group from sugar-based donors to the anthocyanin acceptor molecules.
- **Distribution**: These enzymes are less common and have been identified in only a limited number of plant species. However, recent discoveries suggest their presence may be broader than initially understood.

II-3.2.2 Significance of AATs in anthocyanin biosynthesis

The role of AATs in anthocyanin biosynthesis is pivotal, as acylation:

- **Enhances Structural Diversity**: The introduction of acyl groups contributes to the formation of a wide array of anthocyanin derivatives with unique properties.
- **Stabilizes Coloration**: Acylated anthocyanins exhibit improved stability under various pH conditions, contributing to their vibrant and lasting coloration in plant tissues.
- **Improves Solubility**: Dicarboxylic acylation, in particular, increases the solubility of anthocyanins in aqueous environments, making them functionally versatile.
- **Prevents Degradation**: Acylation protects anthocyanins from enzymatic degradation by microbial glycosidases, ensuring their longevity within plant cells.

II-3.2.3 Research Implications

The molecular and biochemical study of AATs has significant implications for the fields of phytochemistry, biotechnology, and agriculture:

- **Biotechnological Applications**: Understanding AATs enables the development of engineered plants with enhanced pigmentation and stress resilience.
- **Agricultural Enhancements**: Acylation could be harnessed to stabilize anthocyanin content in crops, enhancing their nutritional and aesthetic value.
- **Functional Food Development**: Stable, acylated anthocyanins can be utilized in food industries as natural colorants with improved shelf life and functional benefits.

II-3.3 Acyl-CoA-DependentAATs

II-3.3.1 General aspects of Acyl-CoA-Dependent AAT activities

Acyl-CoA-dependent AATs (Acyltransferases) are enzymes critical for secondary metabolite biosynthesis in plants, particularly in the modification of anthocyanins—pigments responsible for the vibrant colors in flowers, fruits, and other plant parts. These enzymes catalyze the transfer of acyl groups from acyl-CoA donors to acceptor molecules such as anthocyanins, playing a significant role in the structural diversification and functionality of these compounds.

Studies have demonstrated that these enzymes are monomeric proteins with a molecular mass of approximately 50 kDa. Their localization in the cytoplasm or on the cytoplasmic

surface of the endoplasmic reticulum has been confirmed by the absence of transit sequences and immunocytochemical analysis. Optimal enzymatic activity occurs at neutral to slightly alkaline pH (7.0–8.5), conditions compatible with the cytoplasmic environment.

Temporal and spatial regulation of AAT gene expression aligns with anthocyanin biosynthetic processes, particularly in pigmented plant tissues. For instance, transcriptional activators like MYB-related factors (e.g., PAP1 in *Arabidopsis thaliana*) upregulate AAT genes alongside other flavonoid biosynthesis genes. This coordination ensures the production of acylated anthocyanins with distinct physicochemical properties essential for plant survival and reproduction.

II-3.3.2 Acyl donor specificity

AATs can be categorized into **aliphatic** and **aromatic** acyltransferases based on their specificity for acyl-CoA donors:

- **Aliphatic Acyltransferases**: Utilize aliphatic acyl-CoAs, such as malonyl-CoA, acetyl-CoA, or succinyl-CoA. These are predominantly involved in malonylation reactions during anthocyanin biosynthesis.
- **Aromatic Acyltransferases**: Specific to aromatic acyl-CoAs like p-coumaroyl-CoA and caffeoyl-CoA. These enzymes are vital for producing hydroxycinnamoylated anthocyanins, which exhibit enhanced stability and bioactivity.

II-3.3.3 Regiospecificity of Acyl Transfer

Acylation of anthocyanins by AATs occurs with remarkable regiospecificity, meaning the enzyme catalyzes the transfer of the acyl group to a specific position on the anthocyanin molecule. This specificity dictates the structural complexity and functional diversity of the resulting anthocyanins.

For example, in scarlet sage (*Salvia splendens*), sequential acylation events—catalyzed by enzymes like Ss5MaT1 and Ss5MaT2—create salvianin, a dominant pigment. This multistep process involves distinct AATs acting in a coordinated manner, each with unique acyl donor preferences and regiospecificity.

Interestingly, some enzymes, such as Dm3MaT2 from chrysanthemum (*Dendranthema x morifolium*), exhibit the ability to catalyze multiple, consecutive acylation reactions, simplifying the biosynthetic pathway for complex anthocyanins.

II-3.3.4 Acyl acceptor specificity

AATs generally exhibit broad specificity for anthocyanins, capable of acting on various anthocyanidin types (e.g., pelargonidin, cyanidin, and delphinidin). However, the degree and pattern of glycosylation and acylation of substrate anthocyanins can influence enzyme activity. For instance, Ss5MaT1 requires a specific 6′-O-hydroxycinnamyl group for substrate binding, emphasizing the importance of structural motifs in enzymatic specificity.

II-3.3.5 Phylogenetic Insights into AATs

Despite low amino acid sequence similarity (30–60%) among AATs, these enzymes share conserved motifs, such as His-Xaa3-Asp (motif 1) and Asp-Phe-Gly-Trp-Gly (motif 3). These motifs are characteristic of the BAHD superfamily of acyltransferases, which are involved in diverse secondary metabolic processes. The evolutionary conservation of these motifs highlights the functional importance of AATs in plant metabolism.

II-3.4 Serine Carboxypeptidase-like (Scpl)-AATs

Plant secondary metabolism involves numerous pathways leading to the synthesis of specialized metabolites, many of which play crucial roles in plant defense, signaling, and adaptation. One such pathway involves an alternative mechanism for ester formation catalyzed by a specific class of acyltransferases that utilize 1-O-β-glucose esters (1-O-β-acetal esters) as acyl donors, in contrast to the conventional acyl-CoA thioesters. This process represents a significant biochemical innovation in plant metabolism.

II-3.4.1 SCPL Acyl transferases

Acyltransferases involved in this alternative pathway are closely related to serine carboxypeptidases, members of the α/β-hydrolase superfamily. Phylogenetic and sequence analyses have confirmed strong homology, categorizing these enzymes as serine carboxypeptidase-like (SCPL) acyltransferases. These proteins typically possess conserved structural features characteristic of the α/β-hydrolase fold, including a catalytic triad comprising serine, histidine, and aspartate residues. Such structural motifs underpin their catalytic mechanism, which involves a nucleophilic attack by the serine residue to facilitate acyl transfer.

II-3.4.2 Discovery and functional specificity

The enzymatic activity of 1-O-acylglycoside-dependent acyltransferases (AATs) was first identified in cell cultures of *Daucus carota* in 1992. This enzyme facilitates the transfer of acyl groups from hydroxycinnamoyl-1-O-glucosides to the 6th position of the glucose moiety in cyanidin 3-(6"-O-glucosido-2"-O-xylosido) galactoside. A related AAT activity was later discovered in red flowers of carnations (*Dianthus caryophyllus*), where malylated anthocyanins contribute to the pigmentation. In this context, 1-O-malylglucose:pelargonidin 3-O-glucose-6"-O-malyltransferase utilizes 1-O-β-d-malylglucose as the malyl donor, a reaction critical for the biosynthesis of these vibrant pigments.

II-3.4.3 Enzymatic properties and localization

Biochemical studies of SCPL acyltransferases have revealed intriguing properties. For instance, an AAT isolated from the petals of butterfly pea (*Clitoria ternatea*) catalyzes the aromatic acyl transfer from 1-O-acylglucose to anthocyanins, contributing to the biosynthesis of ternatin A1. Molecular cloning of this enzyme revealed it as a presumptive SCPL protein, characterized by an N-terminal signal sequence, three potential N-glycosylation sites, and the canonical Ser-His-Asp catalytic triad.

While the precise cellular localization of SCPL-AATs remains undetermined, evidence suggests a vacuolar localization for some members of this enzyme family. This is in contrast to acyl-CoA-dependent AATs, which are typically cytoplasmic. The vacuolar localization aligns with the compartmentalization of many secondary metabolites, facilitating efficient substrate-channeling and product sequestration.

II-3.4.4 Implications for plant metabolism

The use of 1-O-β-glucose esters as acyl donors represents a divergence from the more commonly studied acyl-CoA thioester pathway. This alternative mechanism may offer evolutionary advantages, such as reduced dependency on CoA pools and specialized regulation of secondary metabolite biosynthesis. Furthermore, SCPL acyltransferases exemplify the functional diversity of the α/β-hydrolase superfamily, highlighting the adaptability of enzyme scaffolds to novel biochemical roles.

II-3.5 Potential Application of AATs

II-3.5.1 Flower Color Modification

The creation of blue flowers in transgenic plants is a complex yet fascinating challenge in the field of phytochemistry. A promising approach involves the accumulation of polyacylated anthocyanins. Anthocyanins are a class of flavonoids responsible for the vivid red, purple, and blue hues in many plant species. The color exhibited by anthocyanins is influenced by their molecular structure, pH, and the presence of co-pigments or metal ions. Polyacylation, specifically with aromatic acids, modifies the chemical structure of anthocyanins to enhance their stability and alter their absorption spectra. These modifications are critical for achieving the desired blue coloration, a color less common in nature compared to red or yellow hues.

To achieve such modifications, genes encoding aromatic acyltransferases (AATs) with diverse substrate specificities play a pivotal role. These enzymes catalyze the acylation of anthocyanins with aromatic acids, creating stable and color-intense compounds. While aliphatic acylation does not directly change the absorption spectra, it significantly improves the stability of anthocyanins by protecting them from degradation due to light, pH fluctuations, or enzymatic breakdown. This increased stability ensures more consistent and long-lasting coloration, which is particularly beneficial for ornamental plants and commercial flowers.

The overexpression of AAT-encoding genes in heterologous plants represents a powerful strategy for anthocyanin modification. This approach allows researchers to introduce epistatic changes, where the interaction between the introduced and endogenous anthocyanins results in novel pigmentation. Such techniques have the potential to revolutionize flower breeding by enabling precise control over flower color.

II-3.5.2 Modification of the functional properties of anthocyanins

Beyond their aesthetic applications, anthocyanins hold immense potential in food science and biomedical fields due to their functional properties. Acylation of anthocyanins has been shown to enhance their biological activities, including antioxidant capacity, bioavailability, and therapeutic potential. Acylation can improve the biological half-life of anthocyanins, making them more stable and effective as nutraceuticals. It also enhances their membrane permeability and intestinal absorption, thereby increasing their efficacy in the human body.

A noteworthy example of regioselective acylation involves the modification of cyanidin 3-O-glucoside, a common anthocyanin. In cultured cells of *Ipomoea batatas* (sweet potato), aromatic AATs facilitate the attachment of aromatic acids to specific positions on the anthocyanin molecule. This regioselectivity is crucial as it determines the resulting compound's stability, solubility, and bioactivity. Such advancements underscore the potential of leveraging enzymatic pathways to produce customized anthocyanin derivatives with enhanced functionality.

III. Biosynthesis of anthocyanins

III-1 Biosynthetic pathway

The biosynthesis of anthocyanins represents a well-characterized branch of the phenylpropanoidpathway, a critical metabolic route in plants for synthesizing secondary metabolites such as flavonoids, lignins, and coumarins. This pathway is highly conserved across plant species and has been extensively studied in model plants like *Arabidopsis thaliana* and agriculturally important crops. The anthocyanin biosynthetic pathway shares its early enzymatic steps with other flavonoid subclasses, including flavones and flavonones, before diverging into specialized branches for anthocyanin production.

III-1.1 Initial steps in the phenylpropanoid pathway

The anthocyanin biosynthetic pathway begins with phenylalanine, an aromatic amino acid derived from the shikimate pathway. Key steps include :

1. Phenylalanine Ammonia-Lyase (PAL): Converts phenylalanine into cinnamic acid by removing an ammonia group. This reaction initiates the phenylpropanoid pathway.
2. Cinnamate-4-Hydroxylase (C4H): Hydroxylates cinnamic acid at the 4-position, producing coumaric acid.
3. 4-Coumaroyl CoA Ligase (4CL): Activates coumaric acid by attaching a Coenzyme A (CoA) moiety, forming 4-coumaroyl CoA.

III-1.2 Flavonoid biosynthesis and branching toward anthocyanins

Following the formation of 4-coumaroyl CoA, the pathway diverges into flavonoid synthesis. The condensation of 4-coumaroyl CoA with malonyl CoA, catalyzed by chalcone synthase (CHS), produces naringenin chalcone. This intermediate is then modified through a series of enzymatic reactions:

1. Chalcone Isomerase (CHI): Converts naringenin chalcone to naringenin, a flavanone.
2. Flavanone 3-Hydroxylase (F3H) and Flavonoid 3′-Hydroxylase (F3′H): Hydroxylate naringenin to produce dihydroflavonols such as dihydrokaempferol and dihydroquercetin, precursors for downstream anthocyanin production.
3. Dihydroflavonol Reductase (DFR): Reduces dihydroflavonols to leucocyanidins, which are immediate precursors to anthocyanidins.
4. Anthocyanidin Synthase (ANS): Oxidizes leucocyanidins to form anthocyanidins like cyanidin, pelargonidin, and delphinidin, which are core anthocyanin structures.

5. UDP- Glucose Flavonoid-3-O-Glycosyltransferase (UFGT) : Glycosylates anthocyanidins to produce stable, water-soluble anthocyanins, which are readily accumulated and utilized by the plant.

III-1.3 Vacuolar transport and storage

Once synthesized in the cytosol, anthocyanins are transported into the vacuole for storage. This transport involves specific proteins and mechanisms:

1. TT12 (Transporter for Flavonoids and Anthocyanins): Encodes a multidrug and toxic compound extrusion (MATE) transporter that facilitates the movement of glycosylated flavonoids and protoanthocyanidins into the vacuole.
2. AHA10 (H+-ATPase): Acidifies the vacuole by establishing a proton gradient, which is hypothesized to provide the energy required for TT12-mediated transport. Mutants of TT12 and AHA10 exhibit transparent testa (seed coat) phenotypes, indicating defects in anthocyanin accumulation.
3. ATP-Binding Cassette (ABC) Transporters: Act in concert with proton antiporters to facilitate anthocyanin sequestration. Glutathione (GSH) conjugates are believed to stabilize anthocyanins during transport.

III-1.4 Biological and agricultural significance

Anthocyanin biosynthesis is vital for plant survival and human nutrition. In plants, anthocyanins serve as antioxidants, UV protectants, and attractants for pollinators. From an agricultural perspective, modulating the anthocyanin pathway can enhance crop aesthetics, nutritional value, and stress resistance. Advances in genetic engineering, such as manipulating TT12 or AHA10 expression, hold potential for optimizing anthocyanin content in economically important crops.

By understanding the detailed mechanics of anthocyanin biosynthesis and transport, researchers can develop strategies to improve the dietary anthocyanin intake and leverage these pigments for agricultural and pharmaceutical applications.

III-2 Modulation of the enzymatic synthesis

III-2.1 Synthesis regulation

The biosynthess of anthocyanins in plant is carefully controlled to respond to developmental and environmental signals. This regulation occurs at various levels, including epigenetic, transcriptional, post-transcriptional and post-translational mechanisms.

III-2.1.1 Epigenetic regulation

Epigenetic modifications influence the accessibility of genes nvolved in anthocyanin biosynthesis:

Histone modifications:

- The tri-methylation of lysine 4 on histone H3 (H3K4me3) is associated with gene activation.
- The histone variant H2A.Z counteracts H3K4me3, repressing biosynthesis genes.
- Mutations affecting the incorporation of H2A.Z increase anthocyanin levels by promoting H3K4me3 at biosynthetic gene loci.

III-2.1.2 Transcriptional regulation

The transcription of anthocyanin biosynthetic genes is primarily mediated by the MBW complex, a ternary structure composed of:

- *MYB Transcription Factors:* Members like MYB75, MYB90, MYB113, and MYB114 positively regulate anthocyanin production.
- *bHLH Proteins:* Such as GLABRA3 (GL3), EGL3, and TT8, which provide specificity to the complex.
- *WD40 Proteins:* TRANSPARENT TESTA GLABRA1 (TTG1) acts as a stabilizing component of the MBW complex.

Certain transcription factors repress anthocyanin synthesis:

- MYB4 and MYB7 bind directly to biosynthetic gene promoters to suppress transcription.
- Proteins like CPC and MYBL2 interfere with MBW complex formation, reducing its activity.

III-2.1.3 Post-Transcriptional regulation

Small regulatory RNAs, such as microRNAs, modulate the stability and translation of mRNAs encoding anthocyanin regulators:

- *miR156:* Reduces the expression of SPL transcription factors, which act as repressors.
- *miR828:* Targets MYB transcription factors, decreasing the expression of biosynthetic genes and anthocyanin levels.
- *miR858a:* Inhibits MYBL2, a repressor, leading to enhanced biosynthesis.

III-2.1.4 Post-Translational regulation

Environmental signals, particularly light, affect the activity and stability of proteins involved in anthocyanin synthesis:

- ***Phosphorylation**:* Light exposure activates MAP kinase-mediated phosphorylation of MYB75, increasing its stability and activity.
- ***Proteasomal Degradation**:* In the absence of light, MYB75 and MYB90 are degraded by the COP1/SPA complex, reducing anthocyanin synthesis. Mutations in these pathways lead to anthocyanin accumulation regardless of light conditions.

III-2.2 Role of synthesis regulation during development and environmental responses

Anthocyanins, water-soluble pigments belonging to the flavonoid group, are synthesized primarily in plants in response to environmental cues and developmental signals. These pigments, responsible for the red, purple, and blue coloration in various plant tissues, serve multiple ecological and physiological functions. Their synthesis is closely associated with light exposure and varies significantly based on plant species, developmental stages, and the specific organ or tissue involved.

III-2.2.1 Role of anthocyanins in attraction and reproduction

Anthocyanins play a crucial ecological role in plant reproduction by attracting animals and pollinating insects. This pigmentation, prominently observed in flowers and the epidermis of fruits, enhances visual appeal, thereby facilitating seed dissemination and pollen transfer. The concentration of anthocyanins in reproductive structures aligns with their developmental stages to optimize their attractiveness during critical periods.

A notable study on blueberry plants elucidated the developmental dynamics of anthocyanin biosynthesis. Early developmental stages were characterized by high levels of procyanidins and quercetin, which diminished as anthocyanin levels rose during fruit maturation. This progression paralleled the expression of key biosynthetic genes, including PAL, CHS, F3H, DFR, and ANS, which peaked during anthocyanin accumulation and subsequently declined. The absence of these genes' expression in white and pink mutants highlighted their indispensability in anthocyanin synthesis. Similar observations were reported in *Antirrhinum majus* (snapdragon), *Petunia hybrida* (petunia), *Pisum sativum* (pea), and *Fragraria ananassa* (strawberry), underscoring the conserved nature of this biosynthetic pathway across diverse species.

III-2.2.2 Anthocyanins and stress responses in plants

As sessile organisms, plants face myriad environmental challenges, necessitating adaptive strategies for survival. Anthocyanins serve as pivotal mediators in plant responses to abiotic stresses, including temperature extremes, water deficits, saline conditions, and high light intensities. Their biosynthesis is finely tuned to these environmental factors, with specific stressors inducing distinct anthocyanin types, suggesting specialized physiological roles.

1. **Temperature Stress:** Low temperatures have been shown to upregulate anthocyanin biosynthesis significantly. For instance, blood oranges stored at 4°C exhibited increased anthocyanin levels alongside heightened expression of related biosynthetic genes compared to those stored at 25°C. Similarly, purple cultivars of Chinese cabbage demonstrated robust anthocyanin accumulation under low temperatures, accompanied by overexpression of transcription factors such as BrMYB2 and BrTT8. In contrast, white cultivars showed overexpression of negative regulators like BrMYBL2.1 and BrLBD38.2, reflecting their diminished capacity for anthocyanin synthesis. Conversely, high temperatures suppress anthocyanin accumulation, downregulating the associated biosynthetic genes.

2. **Drought and Water Stress:** Water scarcity triggers an upregulation of anthocyanin biosynthesis, as observed in grape berries grown under drought conditions. A significant correlation was identified between anthocyanin content and the mRNA levels of biosynthetic genes such as UFGT, CHS, and F3H. Interestingly, drought stress also induces genes involved in brassinosteroid signaling, hinting at a complex interplay between hormonal and environmental signaling pathways. Recent studies propose that the drought-induced accumulation of anthocyanins may be mediated by abscisic acid (ABA) and the regulation of miR156, which modulates downstream targets like SPL13, WD40-1, and DFR.

3. **Saline Stress:** Saline conditions similarly enhance anthocyanin biosynthesis, suggesting their protective role under osmotic stress. While the precise molecular mechanisms remain under investigation, the upregulation of biosynthetic pathways indicates their integral role in maintaining cellular homeostasis.

4. **Light and UV-B Stress:** Exposure to high light intensities or UV-B radiation strongly induces anthocyanin accumulation. These pigments act as photoprotective agents, absorbing excess light and minimizing oxidative damage to cellular components.

III-2.3 Biotechnological approaches to increase anthocyanin levels in food

Although anthocyanins are abundant across the plant kingdom, their concentrations in commonly consumed foods are relatively modest. However, a growing body of evidence highlights their significant health benefits, including protective effects against chronic and degenerative diseases. These discoveries have catalyzed research efforts to better understand the biosynthesis of anthocyanins and to enhance their accumulation in crops widely consumed as part of the human diet.

Focusing on *Solanum lycopersicum* (tomato), an essential crop worldwide, substantial advancement have been made using both classical genetics and modern genetic engineering to increase anthocyanin levels in its fruit. These efforts aim to provide a functional food source with potential health benefits.

III-2.3.1 Classical breeding approaches to enhance anthocyanin content

Classical genetics and conventional breeding have been employed to introduce anthocyanin-accumulating traits into commercial tomato cultivars. This approach involves crossing high-anthocyanin-producing wild species with cultivated tomato varieties. Two key genetic loci, the *Aft* allele (dominant) and the *atv* allele (recessive), have been identified as pivotal contributors to anthocyanin biosynthesis in tomatoes. These alleles were derived from wild species such as *Solanum chilense* and *Solanum cheesmaniae*.

Introducing these alleles led to cultivars like *Indigo Rose* (marketed as “Sun Black"). This variety exhibits high anthocyanin accumulation, but the pigmentation is restricted to the fruit’s skin, leaving the pulp unaffected. Although successful, these efforts have faced limitations in uniformly distributing anthocyanins throughout the fruit.

III-2.3.2 Genetic engineering to boost anthocyanin levels

Modern genetic engineering techniques have surpassed the limitations of classical approaches, enabling more targeted and efficient enhancement of anthocyanin biosynthesis in tomatoes. A landmark study by Butelli and colleagues demonstrated the transformative potential of engineering tomatoes with transcription factors Delila (Del) and Rosea1 (Ros1)

from *Antirrhinum majus* (snapdragon). These modifications resulted in tomatoes with deep purple pigmentation in the skin and pulp, signifying uniform anthocyanin accumulation.

Pilot experiments with Trp53-/- mice predisposed to cancer revealed promising health benefits. Mice fed a diet supplemented with purple tomato powder showed a significant increase in lifespan compared to those fed diets supplemented with conventional red tomato powder or standard diets. These findings highlight the therapeutic potential of anthocyanin-enriched foods in combating degenerative diseases.

III-2.3.3 Implications for agriculture and human health

Enhancing anthocyanin levels in tomatoes offers dual benefits. From an agricultural perspective, anthocyanin-rich tomatoes are more resilient to environmental stressors such as UV radiation and oxidative damage, which can improve crop yield and quality. From a human health perspective, these biofortified tomatoes represent a promising functional food that can potentially mitigate the risk of chronic diseases, such as cardiovascular conditions, diabetes, and certain cancers.

Future research should focus on optimizing these genetic modifications for large-scale agricultural applications while ensuring that these biofortified varieties retain desirable agronomic traits and consumer acceptability. Additionally, exploring synergies between classical breeding and genetic engineering could yield novel tomato cultivars that combine high nutritional value with ecological and economic sustainability.

IV. Anthocyanins in food

IV-1 Natural sources of anthocyanins

Anthocyanins are natural pigments responsible for the red, blue, and purple colors of various fruits and vegetables. Their concentration in plants varies significantly depending on factors such as species, cultivar, growing region, climate, agricultural practices, harvest timing, ripening stage, and conditions of storage and processing. These variations also depend on environmental influences like temperature and light exposure.

Berries are particularly rich in anthocyanins. Common examples include strawberries, blueberries, blackberries, redcurrants, blackcurrants, and raspberries, which typically contain between 100 to 700 milligrams per 100 grams of fresh product. Elderberries and chokeberries stand out for their exceptionally high levels of anthocyanins, ranging from 1.4 to 1.8 grams per 100 grams. Other good sources include cherries, plums, purple corn, pomegranates, eggplants, grapes, red/purple vegetables (like black carrots, red cabbage, and purple cauliflower), and products such as wine. These sources generally provide from a few milligrams to about 200–300 milligrams of anthocyanins per 100 grams.

Recent discoveries have also identified anthocyanins in lesser-known berries such as maqui, myrtle, and açai, which are gaining popularity due to their nutritional properties. Among these compounds, cyanidin is the most prevalent pigment in plants. Cyanidin-3-O-β-glucoside is the most common anthocyanin found in edible plants, followed by delphinidin, pelargonidin, and peonidin derivatives. Structural variations, such as hydroxylation and methylation, influence their color and stability. For instance, hydroxylation tends to create a blue hue but decreases stability, while methylation enhances red tones and increases stability. Glycosylation or acylation can further modify these compounds, affecting their chemical reactivity, polarity, and physical properties.

Fruits are the primary dietary source of anthocyanins, accounting for up to 70% of daily intake, predominantly through apples, pears, berries, stone fruits, and grapes. In some regions, wine significantly contributes to anthocyanin consumption. Although there is no established recommended daily intake for anthocyanins, estimates suggest an average daily intake of about 12.5 milligrams in the United States. In Europe, it ranges from 19 to 65 milligrams for men and 18 to 44 milligrams for women, while in Finland, where berry consumption is higher, it can reach up to 150 milligrams per day.

Anthocyanins are not classified as essential nutrients, but their health benefits are well-documented. They have strong antioxidant properties, which contribute to reducing

inflammation and protecting against chronic and degenerative diseases. Regular consumption of anthocyanin-rich foods has been associated with reduced risks of obesity, diabetes, cardiovascular disease, and certain cancers. Diets rich in fruits and vegetables, such as the Mediterranean diet, are particularly effective in promoting health and longevity. Conversely, inadequate intake of these foods is linked to an increased risk of diseases such as gastrointestinal cancer, ischemic heart disease, and stroke, accounting for millions of deaths globally each year.

Encouraging the consumption of anthocyanin-rich foods is thus vital for supporting overall health and preventing chronic illnesses.

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Encouraging the consumption of anthocyanin-rich foods is thus vital for supporting overall health and preventing chronic illnesses.

IV-2 Anthocyanins as natural food and beverages colorants

The food industry frequently employs chemical substances as colorants, yet these synthetic dyes are associated with significant concerns, particularly regarding health risks. Synthetic dyes have been linked to adverse effects, including potential behavioral and neurological issues. This has led to increased interest in natural alternatives such as anthocyanins, which are not only safe but may also provide health benefits. As a result, anthocyanins are widely approved for use as food colorants in regions such as Europe (designated E163), Japan, the United States, and numerous other countries.

Anthocyanins can be incorporated into a variety of food products, including soft drinks, syrups, jams, jellies, candies, baked goods, dairy products, and powdered formulations. Beyond their role in coloring, anthocyanins offer a dual advantage: they serve as antioxidants, protecting the food from oxidative damage, and enhance the nutritional profile of the products by offering potential health benefits to consumers. Despite these advantages, the use of anthocyanins as natural food colorants faces several challenges.

One major issue is their instability. Anthocyanins are prone to degradation under the influence of light, oxygen, enzymes, metals, pH changes, and temperature fluctuations. This

instability can result in diminished color intensity and reduced effectiveness as antioxidants. Additionally, anthocyanins are more expensive compared to synthetic dyes, making their use less economically viable in certain applications. In some cases, they may also impart undesirable flavors to food, as observed with anthocyanins extracted from sources like red radish.

To address these challenges, various methods have been developed to enhance the stability of anthocyanins. Techniques such as microencapsulation, co-pigmentation with colorless molecules, and chemical modifications like acylation have proven effective. Microencapsulation involves enclosing anthocyanins in protective carriers, such as maltodextrins or beta-glucans, which shield them from environmental factors. These microcarriers can be produced using processes like spray-drying or freeze-drying. Nanoformulations, including nanoliposomes and nanoemulsions, are also being explored for their potential to enhance anthocyanin stability and effectiveness.

Common sources of anthocyanins for use as food colorants include grape skins, radishes, red potatoes, red cabbage, purple sweet potatoes, black carrots, chokeberries, black beans, prunes, and hibiscus flowers. Certain herbs, such as *Thymus moroderi*, have also shown potential. Acylated anthocyanins are particularly valued for their increased stability, making them preferable for industrial applications. However, nonacylated anthocyanins, which can be extracted from fruits like elderberries and chokeberries at a lower cost, also hold promise due to their high availability and cost-effectiveness.

The growing demand for natural and health-promoting ingredients in food products makes anthocyanins an attractive alternative to synthetic dyes. Ongoing advancements in stabilization techniques and cost-reduction strategies are likely to expand their application in the food industry further.

IV-3 Bioavailability of anthocyanins

IV-3.1 Definition and significance of bioavailability

For any biologically active compound to exert its effects, it must reach tissues at concentrations sufficient to trigger a physiological response—a concept termed bioavailability. Despite their high intake through diet, anthocyanins rarely achieve therapeutically active concentrations in plasma. This low bioavailability raises questions about their mode of action and efficacy. Epidemiological and experimental evidence, however, suggests significant health

benefits, indicating that their metabolic pathways and bioactivity may extend beyond simple plasma concentration measurements.

IV-3.2 Mechanisms of absorption and metabolism

IV-3.2.1 Structural influence on absorption

The molecular structure of anthocyanins critically determines their absorption. Simple anthocyanins, such as pelargonidin-based compounds, are absorbed more efficiently compared to those with extensive substituents on the B-ring. This structural simplicity enhances lipophilicity and membrane permeability, facilitating passive diffusion into enterocytes. Additionally, enzymes like lactase phlorizin hydrolase and β-glucosidases contribute to the liberation of aglycones, which are more hydrophobic and readily absorbed.

IV-3.2.2 Gastrointestinal processing

Anthocyanin metabolism begins in the oral cavity, where microbial activity removes glycosidic groups, converting anthocyanins to chalcones. Absorption continues in the stomach and intensifies in the small intestine, the primary site of anthocyanin uptake. Here, anthocyanins encounter transport systems such as the sodium-dependent glucose transporter (SGLT1), facilitating the uptake of glycosides. Acylated anthocyanins, while absorbed, demonstrate significantly lower bioavailability compared to non-acylated forms.

Unabsorbed anthocyanins reach the colon, where they undergo extensive fermentation by the microbiota. Bacteria such as *Bifidobacterium* and *Lactobacillus* metabolize anthocyanins into bioactive phenolic acids (e.g., gallic, protocatechuic, and syringic acids) and simple phenolics (e.g., hydroxytyrosol). These metabolites not only exhibit higher bioavailability but also modulate gut microbiota composition, promoting the proliferation of beneficial bacteria.

IV-3.2.3 Phase I and Phase II Metabolism

After absorption, anthocyanins are subjected to enzymatic modifications, primarily in the liver, but also in renal and enterocyte tissues. Phase I metabolism introduces hydroxyl groups, while Phase II processes, such as glucuronidation, sulfation, and methylation, generate more hydrophilic derivatives. These modifications enhance solubility and facilitate excretion, while also producing intermediates with unique biological activities.

IV-3.3 Modulation by food matrix and processing

The food matrix significantly influences anthocyanin bioavailability. Co-consumption with alcohol, fats, or proteins may enhance or inhibit absorption, depending on their interactions with anthocyanins. Thermal processing, while degrading anthocyanin content, can paradoxically increase bioaccessibility by breaking down the food matrix, releasing bound anthocyanins. Encapsulation techniques, such as microencapsulation and nanoformulation, have also shown promise in enhancing stability and absorption, although their efficacy varies with the encapsulation material and processing methods.

IV-3.4 Interactions with gut microbiota

The interplay between anthocyanins and gut microbiota is reciprocal. Anthocyanins are metabolized by bacterial enzymes into simpler compounds that are more bioavailable and bioactive. Concurrently, these metabolites, along with short-chain fatty acids produced during fermentation, create a favorable gut environment, reducing pH and supporting the growth of probiotic bacteria. This symbiotic relationship underlies many of the observed health benefits of anthocyanins, including improved gut health and systemic anti-inflammatory effects.

The relationship between anthocyanins and the gut microbiota is reciprocal: metabolized by bacterial enzymes into more bioavailable and bioactive compounds, anthocyanins also promote a healthy gut environment by lowering pH and supporting probiotic bacteria. This synergy contributes to their observed health benefits, including improved gut health and systemic anti-inflammatory effects. Regular intake of anthocyanins through a diet rich in fruits and vegetables (such as the Mediterranean diet) is associated with a reduced risk of chronic diseases (cardiovascular conditions, obesity, certain cancers). Despite their low direct bioavailability, their extensive metabolism and interactions with the gut microbiota amplify their systemic effects, underscoring their key role in preventive nutrition..

IV-3.5 Factors influencing daily intake

Global dietary patterns highlight significant variability in anthocyanin consumption (**Table V)**. While berries and red fruits dominate as primary sources, wine contributes substantially in regions like Europe. The absence of a standardized recommended daily intake complicates assessments; however, promoting diverse and anthocyanin-rich diets remains a practical approach to optimizing their health benefits.

Table V. Key factors influencing anthocyanin bioavailability

Factor	Effect on Bioavailability	Details
Molecular Structure	Higher for simpler structures	Pelargonidin-based anthocyanins are more readily absorbed than those with complex substitutions.
Enzymatic Activity	Enhances or modifies absorption	Includes lactase phlorizin hydrolase and β-glucosidases, releasing bioavailable aglycones.
Gut Microbiota	Modifies anthocyanins into bioactive metabolites	Produces phenolic acids and short-chain fatty acids, improving gut health and bioavailability.
Food Matrix	Alters release and absorption	Proteins and fats can enhance bioavailability; fiber may reduce it.
Thermal Processing	Decreases stability but increases bioaccessibility	Heat releases bound anthocyanins but also causes pigment degradation.
Encapsulation	Stabilizes and improves uptake	Methods like nanoformulations enhance absorption but outcomes vary by material and process.

VI- Anthocyanins as food colorants

VI-1 Introduction

Color plays a significant role in the food industry, not just as a visual attribute but also as an important indicator of a product's quality and flavor. Often, the color of food products acts as a "fingerprint" — a distinctive feature that reflects the characteristics of the food, helping consumers to judge its freshness, ripeness, and taste. The visual appeal of food is the first sensory attribute perceived by consumers and is frequently linked to their expectations of quality. Research has shown that food color can directly influence consumer preferences and purchase decisions, with certain hues evoking positive associations related to taste and quality. For example, a rich red color in fruits may be associated with ripeness and sweetness, while vibrant green in vegetables may suggest freshness and nutrient density.

Given its prominence in consumer choice, color stability and enhancement have become major concerns for the food industry. In addition to aesthetic appeal, stability over time — also referred to as "shelf life" — is essential for maintaining the visual attractiveness and, consequently, the marketability of food products. This has led to extensive research by academic and industrial teams seeking to identify new methods of preserving or improving the color stability of foods, especially under various storage conditions, heat treatments, and exposure to light and air. Additionally, the industry's desire to innovate and introduce new products with visually striking and appealing colors has fostered new lines of research focused on developing novel food colorants. These colorants must not only be aesthetically pleasing but also natural and, when possible, health-promoting.

Over the years, there has been a growing trend toward the use of natural food colorants, driven by consumer demand for cleaner labels and a shift away from synthetic additives. This movement seeks to replace artificial colors with plant-derived pigments that offer both functional and aesthetic benefits. The color of food is often closely aligned with its flavor; for example, the yellow of a ripe lemon complements its tartness, and the red of a strawberry matches its sweet flavor. However, some food products deliberately break this convention to create unique or unexpected color-flavor combinations, often targeting younger, trend-conscious consumers. These new color combinations are designed to surprise and intrigue, opening up opportunities for novel food experiences.

As the demand for innovative food colorants continues to rise, the food industry is exploring a variety of sources for these colorants. These can be grouped into three main

categories based on their origin and production methods: natural colorants, nature-identical colorants, and synthetic colorants.

- **Natural colors**: These are derived from plant, animal, or mineral sources and are typically organic compounds. Natural colorants, such as anthocyanins, carotenoids, and betalains, are widely used for their ability to impart vivid, diverse hues. They offer the advantage of being perceived as safer and healthier compared to synthetic options. Additionally, many natural colorants are rich in bioactive compounds, providing not only color but also potential health benefits, including antioxidant and anti-inflammatory properties.
- **Nature-identical colors**: Nature-identical colors are synthesized to chemically replicate the colorants that naturally occur in plants, animals, or other natural sources. For instance, β-carotene and riboflavin are examples of nature-identical colorants. These colorants are produced through chemical synthesis to match the molecular structure of their naturally occurring counterparts. They are considered a bridge between synthetic and natural options, offering some of the same benefits as natural colorants but with improved stability, lower production costs, and easier large-scale manufacturing.
- **Synthetic colors**: Synthetic colorants are man-made compounds that do not occur naturally. Examples include tartrazine and carmoisine, which are widely used in the food industry due to their bright, consistent colors and relatively low production costs. Despite their efficiency and cost-effectiveness, synthetic colorants have been the subject of increasing scrutiny due to potential health concerns, including associations with allergic reactions and behavioral issues, particularly in children. As a result, the use of synthetic colorants is being increasingly regulated, especially in markets where consumer awareness and demand for natural products are high.

The color of food products is far more than just a cosmetic feature; it is closely linked to consumer perception of flavor and quality. The growing trend toward natural, plant-based colorants reflects the increasing consumer preference for products that are perceived as healthier and more sustainable. Phytochemicals, such as anthocyanins, carotenoids, and betalains, are central to the development of natural food colorants, offering both aesthetic appeal and functional health benefits. As research continues to advance in this area, the food industry is poised to explore new ways of harnessing the power of these naturally occurring

compounds to meet the evolving demands of consumers. Furthermore, the ongoing challenge will be to balance innovation, safety, and sustainability, ensuring that the next generation of food colorants is both effective and aligned with consumer values.

VI-2 Anthocyanins as a food colorant

The preference for natural additives over synthetic ones has grown substantially among consumers. This trend is driven by psychological factors; consumers tend to associate natural colorants with health benefits, while synthetic colorants are often linked to toxicity concerns. Consequently, replacing synthetic dyes with natural colorants has become a significant focus within the food industry. Natural pigments exhibit a broad spectrum of colors and are generally regarded as safe. Among these, anthocyanins stand out as crucial components for natural food coloring.

VI-2.1 Anthocyanins as natural colorants

Anthocyanins are water-soluble pigments widely distributed in nature, responsible for the red, purple, and blue hues in many fruits, vegetables, and flowers. Classified as natural colorants under the European Union legislation, anthocyanins are designated as product E163 according to the Codex Alimentarius Commission. Similarly, in the United States, the Food and Drug Administration (FDA) recognizes anthocyanins as natural colors, which do not require certification. Sources for anthocyanins in the U.S. include grape color extracts, grape skin extracts, and various fruit or vegetable juices. However, the extent of their permitted use varies between countries, with the United States generally imposing stricter regulations.

VI-2.2 Chromatic and functional properties of anthocyanins

Anthocyanins exhibit remarkable chromatic properties, making them valuable for a wide range of applications in the food industry. The earliest recorded use of anthocyanin extracts in food dates back to 1879 in Italy, where enocyanin, derived from red grape pomace, was commercialized. Today, grape-derived anthocyanin extracts are commonly used as colorants in sugar confectionery, dairy products, and ice creams, among other applications.

The stability and hue of anthocyanins depend on several factors, including pH, temperature, light exposure, and the presence of co-pigments or metallic ions. These pigments typically display red hues in acidic conditions, blue hues in alkaline environments, and purple

hues in neutral pH ranges. Such pH-dependent chromatic behavior allows for versatile use across various food matrices.

VI-3 Degradation and stability of anthocyanins in food matrixes

Anthocyanins are a class of water-soluble pigments widely distributed in nature, responsible for the red, blue, and purple colors of various fruits, flowers, and vegetables. These compounds have garnered significant attention for their potential as natural colorants in food products, owing to their appealing hues and potential health benefits. However, the stability of anthocyanins during food processing, storage, and extraction is a major challenge. Understanding the factors that influence their degradation and stability is essential for optimizing their use as food colorants.

VI-3.1 Anthocyanin species in aqueous solutions

One of the critical aspects affecting anthocyanin stability is their behavior in solution. Anthocyanins are not present as a single, stable form in aqueous solutions but instead exist in equilibrium between several species, which is highly dependent on the pH of the environment. These species include:

1. **Flavylium cation**: This is the positively charged form of anthocyanins, which is responsible for the red color observed in strongly acidic conditions.
2. **Carbinol base**: At a higher pH, this neutral form of anthocyanins may appear colorless or faintly colored.
3. **Chalcone**: A colorless or slightly colored form, typically resulting from the loss of a hydroxyl group.
4. **Quinonoidal base**: A form that is more likely to occur at alkaline pH, displaying a bluish or violet hue.
5. **Anionic quinonoidal base**: The negatively charged form that can contribute to different shades depending on the pH.

These forms are in constant equilibrium, and the specific distribution of species governs the color of the solution. The total intensity of the red color produced by anthocyanins is observed predominantly at very low pH (pH 1–3), with the flavylium cation being the dominant species. As the pH rises above 3.5, the anthocyanins lose their color intensity, with the equilibrium shifting towards the colorless carbinol base, chalcone, and quinonoidal forms. At

physiological pH (around 7), anthocyanins may show more intense color due to the phenomenon of co-pigmentation, a process where anthocyanins interact with other molecules, enhancing their color.

VI-3.2 Co-pigmentation and its role in color enhancement

Co-pigmentation plays a significant role in enhancing the stability and color intensity of anthocyanins in natural environments. This process can be classified into two types: intramolecular and intermolecular co-pigmentation.

- **Intramolecular co-pigmentation** occurs when acylated anthocyanins, which have hydrophobic acyl groups attached to their structure, stack their acyl moieties against the flavylium nucleus. This interaction stabilizes the anthocyanin structure and results in an increase in color intensity. For example, acylated anthocyanins from the flower Ipomoea tricolor, known for its intense blue color, demonstrate this form of co-pigmentation.
- **Intermolecular co-pigmentation** involves the interaction between anthocyanins and other colorless compounds, such as flavonols, metal ions, or other phenolic compounds. This interaction leads to bathochromic shifts (a shift towards longer wavelengths) in the absorption spectrum of anthocyanins, resulting in a color change, often from red to blue. Metal ions such as magnesium and aluminum are known to interact with anthocyanins and induce this shift, which is particularly important for maintaining color stability in food products.

VI-3.3 Effects of acylation on anthocyanin stability

The acylation of anthocyanins can improve their stability by preventing hydrolysis and enhancing their color stability under various conditions. The acyl group, typically a hydrophobic molecule, can help stabilize the anthocyanin molecule by reducing the susceptibility to hydrolytic degradation. This effect can be attributed to the stacking of the acyl group against the anthocyanin structure, which strengthens the overall molecular configuration. Acylated anthocyanins are often more resistant to environmental stressors such as heat, pH changes, and light exposure. This feature makes acylated anthocyanins particularly valuable as colorants in food applications.

VI-3.4 Temperature and pH influence on anthocyanin stability

Both temperature and pH are critical factors in determining the stability of anthocyanins. At elevated temperatures, the equilibrium of anthocyanin species is displaced towards the colorless carbinol and chalcone forms, resulting in a noticeable loss of color. This phenomenon is especially important in food processing and storage, where high temperatures can lead to significant degradation of anthocyanin pigments. Similarly, the stability of anthocyanins is strongly pH-dependent, as described above. Acidic conditions favor the red coloration, while neutral and alkaline conditions result in the loss of color intensity.

VI-3.5 Sulfur Dioxide (SO_2) and its effect on anthocyanin color stability

Sulfur dioxide (SO_2) is commonly used as a preservative in the food industry, particularly in wine production, to prevent microbial growth and oxidation. However, Sulfur dioxide (SO_2) can also interact with anthocyanins, leading to color fluctuations. When anthocyanins react with SO_2, they form a colorless addition adduct, which reduces the visible color. However, this reaction is reversible, meaning that SO_2 can be released as a gas, allowing the anthocyanin to regain its color. This reversibility is enhanced by heat, which is why the color restoration can occur during food processing.

The ability of anthocyanins to resist SO_2-induced bleaching is an important consideration for their use as food colorants. For example, anthocyanins derived from red grapes are often more resistant to SO_2 bleaching due to the specific positioning of the nucleophilic attack site on the anthocyanin structure, which blocks the reaction.

VI-3.6 Other factors influencing anthocyanin stability

Several other factors can affect the stability and application of anthocyanins as food colorants:

- **Light exposure**: Anthocyanins are sensitive to light, and prolonged exposure can lead to photodegradation, reducing their color intensity and stability.
- **Oxidation**: Oxygen exposure, especially in the presence of metal ions, can catalyze the oxidation of anthocyanins, leading to degradation and loss of color.
- **Microbiological activity**: Enzymes produced by microorganisms can also degrade anthocyanins in food matrixes, further compromising their stability.
- **Solubility and physical form**: The solubility of anthocyanins in the food matrix, as well as their physical form (e.g., powdered, liquid extract), can influence their color intensity and stability.

Additionally, substances such as sugars may enhance the intensity of anthocyanins' color, especially under mildly acidic conditions. However, sugars and their degradation products, such as ascorbic acid, glucose, and fructose, can also catalyze the degradation of anthocyanins, particularly under conditions of high temperature, oxygen exposure, and in the presence of metal ions.

VI-4 Commercial applications of anthocyanins as food colorants

Anthocyanins, as natural pigments, have become widely used in the food industry, particularly as food colorants in various products. These pigments, known for their vibrant red, purple, and blue hues, are extracted from fruits, vegetables, and other plant sources and applied in a range of food products such as soft drinks, fruit preserves, dairy products, and confectionery. However, the use of anthocyanins in food applications requires careful consideration of their stability, pH sensitivity, and susceptibility to environmental factors like light, temperature, and oxidation. This section explores the diverse commercial applications of anthocyanins as food colorants, focusing on their stability, source material, and technological considerations.

VI-4.1 Key applications in food matrixes

VI-4.1.1 Soft drinks

Soft drinks are one of the primary commercial applications for anthocyanins as colorants. Due to their ability to produce vibrant red hues, anthocyanins are used in beverages like fruit sodas, juices, and energy drinks. For these applications, an acidic pH (below 3.5) is required to maintain the red color characteristic of the flavylium cation form of anthocyanins. At higher pH, the color fades, which can result in a less desirable product. The use of anthocyanins in cloudy beverages is problematic because of undesirable side effects such as the “blueing” effect (color shift to blue) and the “thin layer” effect (weakening of color intensity due to light scattering in the solution). These challenges highlight the need for carefully controlled conditions in anthocyanin-based formulations.

VI-4.1.2 Fruit preserves and jams

Anthocyanins are also used in fruit preserves such as jams and canned fruits. These products typically require lower concentrations of anthocyanins (20–60 ppm) to achieve the desired color, which is often red or purple. The presence of sugars in fruit preserves can enhance the intensity of the anthocyanin color, especially under mildly acidic conditions.

However, the challenge of color stability remains, as high temperatures during the processing and storage of these products can shift the equilibrium of anthocyanin species, potentially leading to color loss.

VI-4.1.3 Sugar confectionery and dairy products

In sugar confectionery like jellies, anthocyanins provide vibrant colors. Similarly, dairy products such as yogurt benefit from the natural appeal of anthocyanin-based colorants. The use of anthocyanins in dairy products is more challenging due to the pH and protein interactions that can affect the stability of the pigments. In yogurt, for instance, the anthocyanin may interact with proteins, leading to color instability, which makes careful formulation important to maintain color integrity.

VI-4.1.4 Dry mixes

Anthocyanins are frequently used in powdered products like acid dessert mixes and drink powders. These products benefit from the concentrated form of anthocyanins, and their color can be enhanced by combining the pigments with other ingredients. However, maintaining color stability during storage and rehydration of the dry mixes is critical, and high temperatures, light, and oxidative conditions must be avoided.

VI-4.1.5 Frozen products

The use of anthocyanins in frozen products such as ice cream is less common but is becoming increasingly popular due to consumer demand for natural food colorants. Freezing can affect anthocyanin stability due to changes in the solution's pH and temperature fluctuations, which may cause the pigments to degrade or alter their color intensity. Consequently, stable anthocyanin sources are essential for this application.

VI-4.2 Sources of anthocyanins for food colorants

VI-4.2.1 Grape extracts

Grape extracts are among the most commonly used sources of anthocyanins in the food industry. Grapes, especially red and black varieties, contain a rich assortment of anthocyanins and related compounds, such as flavonols and tannins. These extracts are favored because they exhibit enhanced stability towards pH variations and sulfur dioxide (SO_2) bleaching, which is a common issue in wine production. The complexation of anthocyanins with other compounds in grape extracts also helps to stabilize the color. Furthermore, grapes are abundant and represent a

significant proportion of the global fruit crop, making grape extracts an economically viable source for anthocyanins.

VI-4.2.2 Other fruit sources

Apart from grapes, a variety of other fruits are used to obtain anthocyanin extracts. These include:

- **Blackcurrants**: Known for their deep purple hue, blackcurrants are rich in anthocyanins and have been explored as a source of natural colorants for various food products.
- **Elderberries**: These dark purple berries are another valuable source of anthocyanins used in beverages and preserves.
- **Cranberries and Cherries**: Both fruits are rich in anthocyanins and are used in a variety of food products, including beverages and fruit-based snacks.
- **Açai (Euterpe oleracea)**: Açaí juice, which has a dark purple color, is used in yogurts and smoothies, primarily due to its antioxidant properties in addition to its color.

VI-4.2.3 Vegetable sources

Certain vegetables also provide anthocyanin extracts that are suitable for food coloring applications. These include:

- **Red Cabbage**: Known for its high content of acylated anthocyanins, red cabbage is widely used in food coloring due to its stability toward light, heat, and enzymatic activity.
- **Purple Sweet Potato**: A rich source of anthocyanins, this vegetable extract has been used in various food products, particularly in East Asia.
- **Black Carrot**: Rich in acylated anthocyanins, black carrot extracts are favored for their color stability and resistance to oxidation, making them a good choice for food colorant applications.
- **Red-fleshed Potato and Radish**: These lesser-known vegetable sources also contain anthocyanins and are used to enhance the color of certain food products.

VI-4.2.4 Other potential sources

Anthocyanins can also be obtained from less common sources, such as banana bracts and purple corncobs, which are often considered waste by-products from agricultural processes.

These materials may represent an untapped potential for anthocyanin extraction, particularly as part of sustainability initiatives aimed at reducing food waste.

VI-4.3 Technological considerations in using anthocyanins

VI-4.3.1 Acylation for improved stability

As discussed earlier, acylation of anthocyanins significantly enhances their stability during processing and storage. Acylated anthocyanins are more resistant to degradation from heat, light, and enzymatic activity. This property is particularly important in food applications where these factors cannot always be controlled. Acylation also helps prevent the degradation of anthocyanins under different pH conditions, making them suitable for a wider range of food products.

VI-4.3.2 Resistance to SO_2 bleaching

In food processing, particularly in wine and juice production, the use of sulfur dioxide (SO_2) is common to prevent spoilage and oxidation. However, SO_2 can interact with anthocyanins, causing color loss. Grape extracts, being rich in complex anthocyanin compounds, are more resistant to SO_2 bleaching than simple anthocyanin monomers. This characteristic makes grape-derived anthocyanins particularly useful in applications where SO_2 is used.

VI-4.3.3 Other environmental factors

Other environmental factors, such as exposure to light, oxygen, and high temperatures, can degrade anthocyanins, resulting in a loss of color. This is particularly problematic in food products that are exposed to these conditions during storage. The ability of anthocyanin-rich extracts to resist these factors is a critical consideration for their successful use as food colorants.

VI-5 Future perspectives for the food industry

The growing interest in natural food colorants has driven the food industry to explore the use of anthocyanins—natural pigments found in fruits and vegetables—as alternatives to synthetic dyes. Anthocyanins, known for their vibrant hues, can be used in a variety of food products, including soft drinks, dairy items, pastries, chewing gums, and dry powders.

However, using anthocyanins, especially those derived from food processing by-products, presents challenges that require interdisciplinary research, including food chemistry, biochemistry, nutrition, and toxicology.

VI-5.1 Challenges in using anthocyanins as food colorants

The use of anthocyanin extracts in food formulations is not without its complexities. The food matrix and the intended end-use product play a critical role in determining the formulation and stability of anthocyanin-based colorants. For example, food chemists often have to add other ingredients to improve the solubility and color stability of anthocyanins in various products. Correct formulation, along with adequate processing and storage conditions, is crucial to ensure that the vibrant color remains stable throughout the shelf life of the product.

Moreover, anthocyanins are highly sensitive to factors such as pH, temperature, and light. To address this, additional stabilizers or cross-linking agents may be incorporated into the formulations to prevent degradation. Despite these challenges, the potential for achieving a wide range of stable, attractive colors for different food categories remains strong.

VI-5.2 Appeal of new and unusual food colors

In the modern, highly competitive food market, the appearance of products plays a pivotal role in attracting consumers, particularly younger generations who are more open to innovative and eye-catching food items. Products with unusual colors, such as “funny drinks” or dairy products in shades like orange and blue, are especially appealing to this demographic. While the concept of blue foods may be unappealing to older generations, younger consumers see it as novel and trendy.

Innovations driven by the desire for unique colors in food products are achievable through the use of anthocyanin-derived pigments that display such unusual hues. Anthocyanin-pyruvic acid adducts and portisins are examples of such compounds, which can exhibit vibrant blue or orange shades and are derived from red wine. These pigments can be synthesized in laboratories and classified as nature-identical colors, offering a promising alternative to artificial colorants.

The advantages of these anthocyanin-derived pigments go beyond just their color. Their increased stability, especially with regard to pH fluctuations and resistance to sulfur dioxide (SO_2)-induced discoloration, makes them suitable for use in the food industry, where stability during processing and storage is essential.

VI-5.3 Functional foods: combining color with health benefits

Another growing trend in the food industry is the development of functional foods, which are foods that provide health benefits beyond basic nutrition. According to the European consensus document, functional foods positively affect one or more target functions in the body, improving health or reducing disease risks. Anthocyanins are not only valued for their color but also for their antioxidant and potential health-promoting properties. By replacing synthetic additives with natural colorants like anthocyanins, the food industry can cater to the growing consumer demand for healthier, more natural products.

Consumers increasingly associate natural ingredients with better health outcomes, especially when compared to synthetic additives, which are often linked to concerns about allergies or digestive issues. Therefore, anthocyanins are an attractive option for food manufacturers seeking to meet this demand. As more scientific studies highlight the antioxidant and biological properties of anthocyanins, the public's perception of these compounds as beneficial for health is likely to grow, further increasing their appeal in the food industry.

VI-5.4 Future of anthocyanins in food products

Despite the challenges associated with their use, anthocyanins and their derivatives hold great potential in the food industry. The commercial shift from synthetic to natural colorants is gaining momentum, and the interest in anthocyanin-based colorants is expected to grow significantly in the coming years. However, for this growth to be realized, it is essential to overcome barriers such as the effective extraction, purification, and stabilization of anthocyanins.

Research is already underway to improve industrial-scale extraction and purification processes for anthocyanins. Advances in biotechnology and chemistry, along with collaborations between academia and industry, will likely provide new insights that make the use of anthocyanins more viable for large-scale food production. Additionally, if legislative restrictions on natural extracts are lifted, this could further facilitate the widespread adoption of anthocyanins as food colorants.

VI. Anthocyanins and human health

This section explores the scientific evidence from animal and human clinical studies, examining the impact of anthocyanins and microbial-driven anthocyanin metabolism on cardiovascular and neurodegenerative diseases. With the growing prevalence of these conditions, it is critical to identify effective preventive strategies to slow the progression of age-related diseases. Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality globally, responsible for millions of deaths each year. CVDs include a broad range of heart and blood vessel conditions, such as coronary artery disease, heart attacks, strokes, and hypertension. The burden of CVDs is not only a significant public health concern but also a major economic burden on healthcare systems worldwide.

In parallel, neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and other forms of dementia, have emerged as significant threats to public health, particularly as populations age. Over the past six years, the death toll from neurodegenerative diseases has more than doubled, and they have now risen from the 14th leading cause of death to the 5th, highlighting their increasing impact. Neurodegenerative diseases are characterized by the progressive degeneration of the nervous system's structure and function, leading to a decline in cognitive, motor, and functional abilities. These conditions are often age-related and can severely affect the quality of life of individuals, placing a large emotional and financial strain on patients, caregivers, and healthcare systems.

Emerging evidence suggests that anthocyanins, the water-soluble pigments found in various fruits, vegetables, and plants, may play a significant role in preventing and managing both cardiovascular and neurodegenerative diseases. These compounds are known for their potent antioxidant, anti-inflammatory, and anti-hypertensive properties. Anthocyanins have been shown to exert positive effects on endothelial function, reduce oxidative stress, and modulate vascular inflammation—all of which contribute to improved cardiovascular health. Additionally, the neuroprotective effects of anthocyanins may help protect the brain from age-related cognitive decline, as they can reduce oxidative stress and inflammation in the central nervous system, two key contributors to neurodegenerative diseases.

Interestingly, the beneficial effects of anthocyanins may be enhanced by the microbiota in the gut. Recent studies have highlighted the role of gut microbes in metabolizing anthocyanins into bioactive metabolites, which can then exert systemic health benefits. The transformation of

anthocyanins by gut bacteria into various metabolites is believed to increase their bioavailability and potency, allowing for more pronounced cardiovascular and neuroprotective effects. These microbial-driven metabolites can enter the bloodstream and reach target organs, including the heart and brain, where they may provide additional protection against disease progression.

Given the promising findings from animal models and human clinical studies, there is growing interest in developing anthocyanin-based interventions as part of a broader strategy for preventing and managing cardiovascular and neurodegenerative diseases. Such interventions may include dietary modifications, supplements, or functional foods enriched with anthocyanins or their bioactive metabolites. However, further research is necessary to determine optimal dosages, delivery methods, and long-term effects, as well as to better understand the complex interactions between anthocyanins, gut microbiota, and human health.

As the world's population continues to age, finding effective prevention and intervention strategies for CVDs and neurodegenerative diseases has become more urgent than ever. An exciting area of ongoing research is the potential for anthocyanins, both in their native form and as microbially modified metabolites, to slow or even prevent the progression of these debilitating conditions. The impact of these compounds could be transformative, offering a natural and sustainable approach to improving public health outcomes globally.

VI-1 Antioxidant activity of anthocyanins and human health

Anthocyanins and anthocyanidins belong to the flavonoid polyphenol family and are widely recognized for their remarkable antioxidant properties. In the context of human health, these properties are of paramount importance, as oxidative stress — resulting from an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the organism's antioxidant defense systems — is implicated in the pathogenesis of numerous chronic diseases. Anthocyanins therefore represent a natural line of defense against oxidative insults at the cellular and tissular levels.

From a structural perspective, anthocyanins possess a flavylium scaffold that enables efficient delocalization of radical electrons throughout the molecule, particularly across the sp2 orbitals of the oxonium fragment. The phenolic hydroxyl groups, especially those at *ortho* and *para* positions relative to one another, play a central role in this activity by participating in

semiquinone formation and stabilization of oxidation products, thereby directly contributing to the protection of human cells and tissues against oxidative damage.

Within the organism, anthocyanins exert their protective action primarily through two complementary mechanisms: single electron transfer (SET) and hydrogen atom transfer (HAT). Both pathways enable the efficient neutralization of ROS and RNS responsible for the oxidation of membrane lipids, proteins, and DNA — processes directly associated with cellular aging and the development of chronic pathologies. The best-documented mechanism involves the catechol moieties of anthocyanins (dihydroxyphenyl groups at positions 3' and 4' of the B-ring), which sequentially generate *ortho*-semiquinones and subsequently *ortho*-quinones through two consecutive one-electron transfer reactions, thereby interrupting oxidative chain reactions capable of damaging critical biological structures. The hydroxyl groups at positions 3, 5, and 7 provide additional radical stabilization sites, enabling the formation of pseudo-semiquinone species that evolve into pseudoquinone structures capable of undergoing isomerization via keto-enol tautomerism, further amplifying the overall radical scavenging capacity of these compounds.

The body of accumulated scientific evidence indicates that these antioxidant properties translate into concrete and well-documented protective effects on human health. By reducing LDL lipoprotein oxidation and protecting the vascular endothelium from oxidative insult, anthocyanins contribute to lowering the risk of atherosclerosis and associated cardiovascular diseases. Through their ability to cross the blood-brain barrier and neutralize cerebral ROS, they additionally exert neuroprotective effects against neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Furthermore, by scavenging the free radicals responsible for DNA mutations, anthocyanins exhibit chemopreventive potential against certain types of cancer. Beyond their direct antioxidant action, these compounds also modulate the expression of pro-inflammatory genes and inhibit key enzymes of the inflammatory cascade, including COX-2 and NF-κB, reinforcing their therapeutic relevance in the prevention of chronic inflammatory diseases.

Collectively, these properties position anthocyanins as prime candidates for the development of nutraceuticals and functional food products. Their incorporation into the daily diet, particularly through the consumption of red fruits and berries, represents a natural and accessible strategy for strengthening the organism's antioxidant defenses and reducing the risk of oxidative stress-related diseases.

VI-2 Cardiovascular diseases

Anthocyanins are a group of naturally occurring pigments found in various fruits, vegetables, and plants, which are responsible for the red, blue, and purple colors in many foods. These water-soluble compounds belong to the flavonoid class of polyphenols and have garnered significant attention due to their potent antioxidant, anti-inflammatory, and cardioprotective properties. Anthocyanins are widely studied for their potential health benefits, particularly in the prevention and management of chronic diseases such as cardiovascular diseases (CVDs).

Cardiovascular diseases, which include conditions such as heart disease, stroke, hypertension, and atherosclerosis, are among the leading causes of morbidity and mortality worldwide. These diseases are typically linked to risk factors such as oxidative stress, inflammation, and endothelial dysfunction. Emerging evidence suggests that anthocyanins, through their antioxidant and anti-inflammatory effects, may help protect against these risk factors, thereby contributing to the reduction of cardiovascular disease incidence and progression.

The protective effects of anthocyanins in cardiovascular health are primarily attributed to their ability to combat oxidative stress by neutralizing free radicals, modulate inflammation by inhibiting pro-inflammatory cytokines, and improve endothelial function, which is crucial for vascular health. These effects, combined with their role in improving lipid profiles and reducing blood pressure, make anthocyanins a promising natural compound for supporting heart health.

Incorporating anthocyanin-rich foods into the diet has been associated with improved cardiovascular outcomes, and ongoing research continues to explore the mechanisms through which anthocyanins exert their protective effects. This area of research holds significant promise for developing dietary interventions and functional foods to reduce the burden of cardiovascular diseases globally.

VI-2.1 In vivo evidence for the cardioprotective effects of anthocyanins

Chronic inflammation of the vascular endothelium is a central driver of atherosclerosis and its associated cardiovascular complications, including hypertension, coronary artery disease, and ischemic stroke. The apoE-deficient mouse model, which spontaneously develops severe hypercholesterolemia and atherosclerotic lesions, has been instrumental in elucidating the cardioprotective mechanisms of anthocyanins at multiple stages of atherogenesis.

Anthocyanins attenuate the early steps of plaque formation by enhancing eNOS phosphorylation and preserving nitric oxide bioavailability, thereby maintaining endothelial integrity. They concurrently suppress endothelial activation by downregulating the expression of adhesion molecules VCAM-1 and ICAM-1, reducing monocyte recruitment to sites of oxidized LDL accumulation. At the oxidative level, anthocyanins from sources including purple sweet potato and bilberry significantly decrease ROS production, lipid peroxidation markers, and pro-inflammatory gene expression, while modulating hepatic lipid metabolism to reduce circulating cholesterol levels. Foam cell formation — a hallmark of advanced atherosclerosis — is further limited through inhibition of macrophage oxLDL uptake and upregulation of ABCG1-mediated cholesterol efflux, promoting lesion regression.

These anti-atherogenic effects have been consistently demonstrated across multiple animal models, including mice, rats, rabbits, and zebrafish, with anthocyanins from Concord grape, cornelian cherry, acai, and grape skin reducing LDL oxidation, serum triglycerides, and aortic lesion burden. Beyond atherosclerosis, anthocyanins exhibit protective effects against cardiac hypertrophy and fibrosis, as evidenced by roselle extract ameliorating cardiac oxidative stress in obesity models and grape-derived anthocyanins improving vascular function and reducing blood pressure in hypertensive rats. Collectively, these findings establish a robust preclinical rationale for the therapeutic use of anthocyanins in cardiovascular disease prevention.

VI-2.2 Clinical evidence for anthocyanin cardioprotection

Cardiovascular diseases (CVDs) remain a leading cause of global morbidity and mortality, with atherosclerosis — a chronic inflammatory condition of the arterial walls — representing a primary underlying pathology. Anthocyanins, natural polyphenolic pigments found in fruits and vegetables, have demonstrated promising cardioprotective effects in both preclinical and clinical settings, targeting multiple stages of CVD pathogenesis through complementary mechanisms.

At the vascular level, anthocyanins attenuate endothelial dysfunction by reducing the expression of key adhesion molecules, including VCAM-1 and ICAM-1, thereby limiting monocyte recruitment and subsequent foam cell formation. Concurrently, they suppress systemic inflammation by downregulating pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and CCL2, particularly in overweight and obese individuals. With respect to lipid metabolism, randomized controlled trials consistently report reductions in LDL cholesterol and triglyceride levels, alongside improvements in HDL functionality, including enhanced

antioxidant capacity and cholesterol efflux — both critical to plaque regression and reverse cholesterol transport. Their antioxidant action is further mediated through upregulation of paraoxonase 1 (PON1) activity and inhibition of inducible nitric oxide synthase (iNOS), collectively reducing oxidative modification of lipoproteins and endothelial injury.

Clinical trials additionally highlight the antihypertensive properties of anthocyanins, with boysenberry juice and roselle extract demonstrating significant reductions in systolic blood pressure, the latter exhibiting effects comparable to pharmaceutical agents such as lisinopril. Anthocyanin-rich interventions have also been shown to improve postprandial glycemic responses and insulin sensitivity in patients with impaired glucose tolerance or type 2 diabetes, further mitigating cardiovascular risk. Notably, a 24-week dose-response trial confirmed that supplementation at 320 mg/day significantly improved lipid profiles and reduced inflammatory biomarkers including CRP and IL-1β.

Despite this body of evidence, variability in clinical outcomes — particularly regarding lipid-modulating effects in healthy populations — underscores the need for standardized protocols with respect to anthocyanin source, dosage, and intervention duration, in order to establish robust therapeutic guidelines for their use in cardiovascular disease prevention.

VI-3 Neurodegenerative diseases

Neurodegenerative diseases, including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), prion diseases, and amyotrophic lateral sclerosis (ALS), represent a diverse group of disorders characterized by the abnormal accumulation of misfolded or unfolded proteins, either inside or outside neurons. These proteins typically aggregate into toxic forms that disrupt normal cellular function and contribute to neuronal death. As the global population continues to age, the incidence of neurodegenerative diseases is rising, making these conditions a significant public health concern. The progression of these diseases often leads to severe cognitive, motor, and functional impairments, significantly impacting the quality of life for affected individuals.

The pathogenesis of neurodegenerative diseases is multifactorial, involving a complex interplay of molecular and cellular mechanisms. Key pathways implicated in these diseases include apoptosis (programmed cell death), autophagy (the cellular process of degrading and recycling damaged components), mitochondrial dysfunction, and oxidative DNA damage and repair. Each of these pathways can contribute to neuronal damage, but the specific functional context in which they operate varies across different neurodegenerative diseases. For example,

in Alzheimer's disease, the accumulation of amyloid-beta plaques and tau tangles disrupts neuronal communication, while in Parkinson's disease, the loss of dopaminergic neurons due to the accumulation of misfolded alpha-synuclein is a key feature. Despite extensive research, the precise mechanisms underlying these diseases remain unclear, which presents a major challenge for the development of effective therapies.

One of the most pressing challenges in neurodegenerative disease research is identifying therapeutic strategies that can effectively slow disease progression or prevent the onset of symptoms. While pharmacological treatments are currently available for some neurodegenerative diseases, they often only provide limited symptomatic relief and do not address the underlying causes of the diseases. Thus, there is growing interest in exploring natural compounds with neuroprotective properties, and anthocyanins have emerged as a promising candidate in this regard.

Anthocyanins, a group of flavonoid compounds found in various fruits and vegetables, have garnered attention due to their potent antioxidant, anti-inflammatory, and neuroprotective properties. These compounds have been shown to protect neurons from oxidative stress, a key contributor to neuronal damage in neurodegenerative diseases. By scavenging free radicals and reducing oxidative damage, anthocyanins can help maintain cellular homeostasis and support neuronal health. In addition to their antioxidant effects, anthocyanins have also been found to modulate neuroinflammation, a common feature of neurodegenerative diseases. Chronic inflammation in the brain can exacerbate neuronal damage, but anthocyanins can suppress the activation of inflammatory pathways, thereby reducing the inflammatory response and potentially slowing disease progression.

Furthermore, anthocyanins have been shown to modulate various cell signaling pathways involved in cell survival, protein degradation, and stress response. For example, they may influence the autophagy pathway, which clears damaged proteins and organelles from cells. By enhancing the efficiency of autophagy, anthocyanins may help reduce the accumulation of toxic proteins that contribute to neurodegeneration. Additionally, anthocyanins can regulate mitochondrial function, helping to maintain energy production and reduce the generation of reactive oxygen species (ROS) that can damage cellular components.

Given their ability to address multiple aspects of neurodegenerative disease pathology, anthocyanins are being investigated as potential preventive and therapeutic agents. Clinical and preclinical studies have demonstrated the neuroprotective effects of anthocyanins in animal models of Alzheimer's disease, Parkinson's disease, and other neurodegenerative conditions.

These studies have highlighted the potential of anthocyanins to protect neurons from oxidative stress and inflammation and improve cognitive function and motor coordination in disease models.

While the evidence supporting the neuroprotective effects of anthocyanins is promising, further research is needed to understand better their mechanisms of action, bioavailability, and optimal dosages for therapeutic use. Additionally, human clinical trials are necessary to confirm the efficacy of anthocyanins as a viable therapeutic strategy for neurodegenerative diseases. Nonetheless, the growing body of evidence suggests that anthocyanins, as natural compounds with broad biological activity, may hold significant promise as part of a comprehensive approach to preventing and managing neurodegenerative diseases.

VI-3.1 In vivo evidence for the neuroprotection effects of anthocyanins

The brain is particularly susceptible to oxidative stress due to its high metabolic rate and oxygen demand, utilizing more oxygen than any other organ. This elevated oxygen consumption renders neural tissues prone to the production of reactive oxygen species (ROS). Additionally, the brain is rich in redox-active metals, such as copper and iron, which further exacerbate ROS generation. The cellular membranes in the brain, composed predominantly of polyunsaturated fatty acids, are highly vulnerable to lipid peroxidation, a key process in oxidative damage.

Regulation of intracellular ROS metabolism involves numerous proteins that function within redox signaling pathways. Among these, the phosphatidyl-inositol-3-kinase (PI3K)/AKT pathway is pivotal. ROS-mediated modulation of this pathway is critical for cellular processes such as senescence and apoptosis. Dysregulation in this system is often implicated in neurodegenerative diseases, including Alzheimer’s disease (AD).

VI-3.1.1 Anthocyanins and their role in neuroprotection and inflammation

Anthocyanins, exhibit significant neuroprotective and anti-inflammatory properties, making them promising candidates in the prevention and management of neurodegenerative and neuroinflammatory disorders. Their protective effects operate through multiple complementary mechanisms targeting both oxidative stress and inflammatory signaling pathways.

From a neuroprotective standpoint, anthocyanins have been shown to mitigate neurodegeneration in transgenic mouse models of Alzheimer's disease (APP/PS1) by reducing ROS levels, inhibiting apoptosis, and modulating the PI3K/Akt/GSK3 signaling cascade. These

compounds further activate the Nrf2/HO-1 endogenous antioxidant pathway, upregulating cytoprotective genes essential for neuronal survival. Advanced delivery strategies, such as anthocyanin-loaded PEG-gold nanoparticles, have been shown to extend these benefits by preventing tau protein hyperphosphorylation — a defining hallmark of Alzheimer's disease pathology. At the cellular level, anthocyanins consistently reduce oxidative damage biomarkers, most notably malondialdehyde (MDA), while enhancing the activity of key endogenous antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx), thereby preserving redox homeostasis within neuronal tissues.

Regarding their anti-inflammatory role, anthocyanins modulate critical neuroinflammatory pathways by suppressing microglial inflammasome activation and downregulating JNK signaling. This translates into a marked reduction in the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which are key mediators of neuroinflammation and contributors to progressive neuronal damage. Collectively, these antioxidant and anti-inflammatory actions position anthocyanins as compelling bioactive agents for the development of neuroprotective nutraceuticals and therapeutic strategies targeting oxidative stress-driven neurological conditions.

VI-3.1.2 Anthocyanins in high-fat diet models

Given the association between high-fat diets and increased risk of neurodegenerative diseases, anthocyanins have been investigated for their protective effects in such conditions. Dietary supplementation with blackberry extracts and purple sweet potato pigments has been shown to counteract neuroinflammation and oxidative stress induced by high-fat diets. These effects include reductions in pro-inflammatory cytokines and improved neuronal function, as evidenced by enhanced neuroplasticity and behavioral outcomes in memory tests.

VI-3.1.3 Memory and cognitive benefits

Anthocyanins significantly improve cognitive functions, including memory and learning, as shown in various behavioral tests such as the Morris water maze, Y-maze, and novel object recognition tests. Additionally, motor coordination improvements have been observed in studies employing the rotating rod and open-field tests. Molecular studies, including Western blot analyses, reveal that anthocyanins upregulate presynaptic and postsynaptic markers associated with synaptic plasticity, further supporting their role in cognitive enhancement.

IV-3.2 Clinical studies

VI-3.2.1 Experimental evidence for neuroprotective role of anthocyanins

Clinical and preclinical studies collectively provide compelling evidence that anthocyanins exert meaningful neuroprotective effects through the convergent modulation of oxidative stress, neuroinflammation, and cognitive function. By targeting redox-sensitive pathways — including the Nrf2/HO-1 axis and the PI3K/Akt/GSK3 cascade — anthocyanins reinforce endogenous antioxidant defenses, reduce ROS accumulation, and restore redox homeostasis in neuronal tissues, thereby limiting oxidative damage to lipids, proteins, and DNA.

In experimental models of neurodegenerative diseases, including Alzheimer's and Parkinson's disease, anthocyanins have consistently demonstrated the ability to attenuate hallmark pathological features such as amyloid-β aggregation, tau hyperphosphorylation, and dopaminergic neuronal loss. These effects are further supported by their capacity to suppress neuroinflammatory cascades, notably through inhibition of microglial NF-κB and NLRP3 inflammasome activation, resulting in reduced expression of pro-inflammatory mediators including TNF-α, IL-6, and IL-1β — cytokines critically involved in the progression of neurodegeneration.

At the functional level, both animal studies and human clinical trials report that regular anthocyanin consumption is associated with measurable improvements in cognitive performance, memory consolidation, and behavioral outcomes. These findings, supported by biomarker evidence including reduced MDA levels and enhanced SOD and GPx activity, underscore the therapeutic relevance of anthocyanins as nutraceutical agents in strategies aimed at preventing or slowing the progression of oxidative stress-driven neurological disorders.

VI -3.2.2 Cognitive function and behavioral outcomes

The cognitive benefits of anthocyanins extend beyond their neuroprotective properties. In human studies, anthocyanin supplementation has been associated with improved verbal fluency, short-term memory, and long-term memory in elderly individuals with mild-to-moderate dementia. For example, daily consumption of cherry juice improved memory-related outcomes without significantly altering inflammatory markers like C-reactive protein (CRP) or interleukin-6 (IL-6). Similarly, interventions with Concord grape juice and wild blueberry juice have shown promise in enhancing cognitive function in individuals with early memory decline. Functional magnetic resonance imaging (fMRI) studies further corroborate these findings,

revealing enhanced neural responses in brain regions associated with working memory following anthocyanin supplementation.

In younger populations, the effects of anthocyanins on cognition are more variable. While some studies report no significant benefits, others suggest improved cognitive performance, particularly in children aged 7–10 years, following anthocyanin supplementation.

VI-3.2.3 Neuroprotection in metabolic and diet-induced stress

High-fat diets, known to increase the risk of neurodegenerative diseases, provide a relevant model for studying the neuroprotective effects of anthocyanins. In such contexts, anthocyanin-rich extracts have been shown to counteract diet-induced neuroinflammation and oxidative stress. For example, purple sweet potato color treatment mitigates hippocampal apoptosis by enhancing AMP-activated protein kinase-mediated autophagy and restoring neurotrophic factors. These interventions also reduce the expression of inflammatory markers, further highlighting the multifaceted role of anthocyanins in preserving brain health under metabolic stress.

VI-3.2.4 Mechanistic insights and future directions

Anthocyanins exert their neuroprotective effects through a variety of mechanisms, including the regulation of redox balance, suppression of inflammation, and inhibition of neurotoxic protein aggregation. Their ability to improve cognitive function is supported by behavioral assessments such as the Morris water maze, Y-maze, and novel Object recognition tests. Additionally, anthocyanins enhance motor coordination, as evidenced by rotating rods and open-field tests.

While the evidence for anthocyanins' neuroprotective and cognitive benefits is compelling, further research is needed to determine the optimal dosing regimens and treatment durations for human populations. Longitudinal studies and randomized clinical trials will be critical in translating these findings into effective dietary or therapeutic interventions.

Conclusion

Anthocyanins are a group of naturally occurring, water-soluble pigments widely distributed in the plant kingdom, contributing to many fruits, vegetables, and flowers' red, purple, and blue colors. These compounds have gained significant attention due to their wide array of beneficial effects on human health, particularly in preventing and managing various diseases. Studies have shown that an adequate daily intake of anthocyanins may offer protection against numerous health conditions, including neurodegenerative diseases and cardiovascular diseases (CVDs), which are among the leading causes of morbidity and mortality globally.

The chemistry and biochemistry of anthocyanins have been extensively studied in recent years better to understand their structure, stability, and potential applications. Researchers have made significant strides in enhancing the stability of anthocyanins, as their natural form is often prone to degradation due to factors like light, heat, and pH. This instability has limited their use in certain applications, particularly in the food industry, where anthocyanins are primarily used as natural colorants and nutritional additives. To overcome these challenges, new approaches such as nanoformulations and encapsulation techniques have been developed. These methods help protect anthocyanins from degradation, ensuring that their health benefits are maintained over time. Furthermore, chemical modifications, such as acylation, have been explored to create more stable versions of anthocyanins. These modifications enhance their stability and may improve their bioavailability, which refers to the extent and rate at which these compounds are absorbed and utilized by the body.

Improving the bioavailability of anthocyanins is crucial to maximizing their health benefits. When anthocyanins are consumed naturally, they often undergo rapid metabolism in the digestive system, reducing the amount that reaches the bloodstream and target tissues. By employing innovative delivery systems like nanoencapsulation, researchers aim to protect anthocyanins from this rapid metabolism, ensuring that a larger amount of the compound is available to exert its beneficial effects. Such formulations may also provide a controlled release of anthocyanins, allowing for sustained activity in the body further enhancing their therapeutic potential.

The protective effects of anthocyanins in cardiovascular and neurodegenerative diseases appear closely related to their antioxidant and anti-inflammatory properties. Oxidative stress and chronic inflammation are key contributors to the pathogenesis of these diseases. In CVDs,

oxidative stress accelerates the formation of plaques in blood vessels, leading to conditions such as atherosclerosis, while in neurodegenerative diseases, oxidative damage to neurons is a major factor in disease progression. Anthocyanins, through their potent antioxidant activity, help neutralize free radicals and reactive oxygen species (ROS), reducing oxidative stress and limiting cellular damage. Furthermore, their anti-inflammatory properties help modulate immune responses and prevent the chronic inflammation that exacerbates tissue damage in these diseases.

Given the overlapping mechanisms in the pathophysiology of cardiovascular and neurodegenerative diseases, it may be beneficial to study anthocyanins in models that address both conditions simultaneously. One such model is the apoE-deficient mouse, a widely used animal model of human atherosclerosis. These mice develop hyperlipidemia and exhibit all the major lesions associated with human atherosclerosis, making them a valuable tool for studying cardiovascular disease. Interestingly, the same model is also used to study Alzheimer's disease (AD), a neurodegenerative disorder characterized by the accumulation of amyloid plaques and tau tangles in the brain. This dual use of the apoE-deficient mouse model provides a unique opportunity to investigate the potential of anthocyanins in preventing or mitigating the effects of both CVDs and neurodegenerative diseases in the same experimental framework.

In conclusion, anthocyanins are promising as natural compounds with many health benefits. By improving their stability, bioavailability, and understanding their mechanisms of action, anthocyanins could play a key role in preventing and managing age-related diseases such as cardiovascular diseases and neurodegenerative disorders. Further research is needed to fully elucidate the molecular mechanisms through which anthocyanins exert their protective effects and identify the most effective formulations and dosages for human use. With continued advancements in these areas, anthocyanins may become a cornerstone of dietary strategies aimed at improving health and reducing the burden of chronic diseases.

References

Aliaño-González, M. J., Ferreiro-González, M., Espada-Bellido, E., Carrera, C., Palma, M., Álvarez, J. A., ... & F. Barbero, G. (2020). Extraction of anthocyanins and total phenolic compounds from açai (euterpe oleracea mart.) using an experimental design methodology. part 1: Pressurized liquid extraction. *Agronomy*, *10*(2), 183.

Antonio-Gomez, M. V., Salinas-Moreno, Y., Hernández-Rosas, F., Martínez-Bustos, F., Andrade-González, I., & Herrera-Corredor, J. A. (2021). Optimized extraction, microencapsulation, and stability of anthocyanins from Ardisia compressa K. fruit. *Polish Journal of Food and Nutrition Sciences*, *71*(3), 299-310.

Batista, A. G., Mendonca, M. C. P., Soares, E. S., da Silva-Maia, J. K., Dionísio, A. P., Sartori, C. R., & Júnior, M. R. M. (2020). Syzygium malaccense fruit supplementation protects mice brain against high-fat diet impairment and improves cognitive functions. *Journal of functional foods*, *65*, 103745

Cai, H., Zhang, M., Chai, M., He, Q., Huang, X., Zhao, L., & Qin, Y. (2019). Epigenetic regulation of anthocyanin biosynthesis by an antagonistic interaction between H2A. Z and H3K4me3. *New Phytologist*, *221*(1), 295-308.

Chen, B. H., & Stephen Inbaraj, B. (2019). Nanoemulsion and nanoliposome based strategies for improving anthocyanin stability and bioavailability. *Nutrients*, *11*(5), 1052.

Chua, L. S., Thong, H. Y., & Soo, J. (2024). Effect of pH on the extraction and stability of anthocyanins from jaboticaba berries. *Food Chemistry Advances*, *5*, 100835.

Cunha, R., Trigueiro, P., Orta Cuevas, M. D. M., Medina-Carrasco, S., Duarte, T. M., Honório, L. M. D. C., ... & Osajima, J. A. (2023). The stability of Anthocyanins and their derivatives through clay minerals: revising the current literature. *Minerals*, *13*(2), 268.

Custodio-Mendoza, J. A., Aktaş, H., Zalewska, M., Wyrwisz, J., & Kurek, M. A. (2024). A review of quantitative and topical analysis of anthocyanins in food. *Molecules*, *29*(8), 1735.

Eker, M. E., Aaby, K., Budic-Leto, I., Rimac Brnčić, S., El, S. N., Karakaya, S., ... & de Pascual-Teresa, S. (2019). A review of factors affecting anthocyanin bioavailability: Possible implications for the inter-individual variability. *Foods*, *9*(1), 2.

ElShamey, E. A., Yang, X., Yang, J., Pu, X., Yang, L. E., Ke, C., & Zeng, Y. (2025). Occurrence, biosynthesis, and health benefits of anthocyanins in rice and barley. *International Journal of Molecular Sciences*, *26*(13), 6225.

Feyissa, B. A., Arshad, M., Gruber, M. Y., Kohalmi, S. E., & Hannoufa, A. (2019). The interplay between miR156/SPL13 and DFR/WD40–1 regulate drought tolerance in alfalfa. *BMC plant biology*, *19*(1), 434.

Ge, J., Yue, X., Wang, S., Chi, J., Liang, J., Sun, Y., ... & Yue, P. (2019). Nanocomplexes composed of chitosan derivatives and β-Lactoglobulin as a carrier for anthocyanins: Preparation, stability and bioavailability in vitro. *Food Research International*, *116*, 336-345.

Igwe, E. O., Charlton, K. E., & Probst, Y. C. (2019). Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults. *Journal of Human Nutrition and Dietetics*, *32*(5), 578-590.

Khan, M.S.; Ali, T.; Kim, M.W.; Jo, M.H.; Chung, J.I.; Kim, M.O. (2019). Anthocyanins improve hippocampus-dependent memory function and prevent neurodegeneration via JNK/Akt/GSK3β signaling in LPS-treated adult mice. Mol. Neurobiol. 2019, 56, 671–687.

Khezri, S., Ghanbarzadeh, B., & Ehsani, A. (2025). Barberry anthocyanins: recent advances in extraction, stability, biological activities, and utilisation in food systems—a review. *International Journal of Food Science and Technology*, *60*(1), vvaf031.

Khoo, H. E., Ng, H. S., Yap, W. S., Goh, H. J. H., & Yim, H. S. (2019). Nutrients for prevention of macular degeneration and eye-related diseases. *Antioxidants*, *8*(4), 85.

Lavefve, L., Howard, L. R., & Carbonero, F. (2020). Berry polyphenols metabolism and impact on human gut microbiota and health. *Food & function*, *11*(1), 45-65.

Lourith, N., & Kanlayavattanakul, M. (2020). Improved Stability of Butterfly Pea Anthocyanins with Biopolymeric Walls. *Journal of Cosmetic Science*, *71*(1). 1–10

Lu, Z., Wang, X., Lin, X., Mostafa, S., Zou, H., Wang, L., & Jin, B. (2024). Plant anthocyanins: Classification, biosynthesis, regulation, bioactivity, and health benefits. *Plant Physiology and Biochemistry*, *217*, 109268.

Maaz, M., Sultan, M. T., Noman, A. M., Zafar, S., Tariq, N., Hussain, M., ... & Al Abdulmonem, W. (2025). Anthocyanins: From Natural Colorants to Potent Anticancer Agents. *Food Science & Nutrition*, *13*(5), e70232.

Moura, C. D., do Carmo, M. A. V., Xu, Y. Q., Azevedo, L., & Granato, D. (2024). Anthocyanin-rich extract from purple tea: Chemical stability, cellular antioxidant activity, and protection of human plasma. *Current Research in Food Science*, *8*, 100701.

Ndoye, B., Mbow, B., Faye, P. G., Kane, C., Cissé, M., & Ayessou, N. (2025). An overview of the extraction methods of anthocyanins from local African roselle and their use as potential functional foods and food additives colorants. *Agriculture and Food Bioactive Compounds*, *2*(1), 15-31.

Noman, A. M., Sultan, M. T., Maaz, M., Mazhar, A., Tariq, N., Imran, M., ... & Al Jbawi, E. (2025). Nutraceutical Potential of Anthocyanins: A Comprehensive Treatise. *Food Science & Nutrition*, *13*(5), e70164.

Oleinits, E., Hatem, M. A., Deineka, V., Chulkov, A., Blinova, I., & Tretiakov, M. (2019). Determination of anthocyanins of purple carrot two cultivars. In *1st International Symposium Innovations in Life Sciences (ISILS 2019)* (pp. 333-336). Atlantis Press.

Ongkowijoyo, P., Luna-Vital, D. A., & de Mejia, E. G. (2018). Extraction techniques and analysis of anthocyanins from food sources by mass spectrometry: An update. *Food chemistry*, *250*, 113-126.

Ötleş, S., & Nakilcioglu, E. (2025). Health Effects of Anthocyanins. In *Handbook of Analysis and Extraction Methods of Anthocyanins* (pp. 114-134). CRC Press.

Park, S., Cho, S. M., Jin, B. R., Yang, H. J., & Yi, Q. J. (2019). Mixture of blackberry leaf and fruit extracts alleviates non-alcoholic steatosis, enhances intestinal integrity, and increases Lactobacillus and Akkermansia in rats. *Experimental Biology and Medicine*, *244*(18), 1629-1641.

Pieczykolan, E., & Kurek, M. A. (2019). Use of guar gum, gum arabic, pectin, beta-glucan and inulin for microencapsulation of anthocyanins from chokeberry. *International Journal of Biological Macromolecules*, *129*, 665-671.

Rodriguez-Amaya, D. B. (2019). Update on natural food pigments-A mini-review on carotenoids, anthocyanins, and betalains. *Food Research International*, *124*, 200-205

Sadowska-Bartosz, I., & Bartosz, G. (2024). Antioxidant activity of anthocyanins and anthocyanidins: a critical review. *International journal of molecular sciences*, *25*(22), 12001.

Shi, M., Mathai, M. L., Xu, G., McAinch, A. J., & Su, X. Q. (2019). The effects of supplementation with blueberry, cyanidin-3-O-β-glucoside, yoghurt and its peptides on obesity and related comorbidities in a diet-induced obese mouse model. *Journal of functional foods*, *56*, 92-101.

Smerea, O., & Bulgaru, V. (2024). Anthocyanins–methods of extraction, stabilization and applications. *Journal of Engineering Sciences*, (3), 179-195.

Tena, N., & Asuero, A. G. (2022). Up-to-date analysis of the extraction methods for anthocyanins: Principles of the techniques, optimization, technical progress, and industrial application. *Antioxidants*, *11*(2), 286.

Tian, L., Tan, Y., Chen, G., Wang, G., Sun, J., Ou, S., ... & Bai, W. (2019). Metabolism of anthocyanins and consequent effects on the gut microbiota. *Critical Reviews in Food Science and Nutrition*, *59*(6), 982-991.

V. González de Peredo, A., Vázquez-Espinosa, M., Espada-Bellido, E., Ferreiro-González, M., Amores-Arrocha, A., Palma, M., ... & Jiménez-Cantizano, A. (2019). Alternative ultrasound-assisted method for the extraction of the bioactive compounds present in myrtle (Myrtus communis L.). *Molecules*, *24*(5), 882.

Voet, S., Srinivasan, S., Lamkanfi, M., & van Loo, G. (2019). Inflammasomes in neuroinflammatory and neurodegenerative diseases. *EMBO molecular medicine*, *11*(6), e10248.

Xu, Z., Xie, J., Zhang, H., Pang, J., Li, Q., Wang, X., ... & Ling, W. (2021). Anthocyanin supplementation at different doses improves cholesterol efflux capacity in subjects with dyslipidemia—A randomized controlled trial. *European journal of clinical nutrition*, *75*(2), 345-354.

Zhang, L., Wang, Y., Cao, Y., Wang, F., & Li, F. (2025). Enhancing the bioavailability and stability of anthocyanins for the prevention and treatment of central nervous system-related diseases. *Foods*, *14*(14), 2420.