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By:

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Title:

Optimization of Natural Anthocyanin Extraction from Onion Peels

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بسم الله الرحمن الرحيم

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Dedication

To all those who hold a special place in my heart those to whom I owe every step of this journey:

To Allah, the Most Merciful, who granted me the strength, faith, and answered prayers without You, none of this would have been possible.

To the man who once called me the apple of his eye, who sees in my smallest achievement his greatest pride my father, Amara. Your support and love are my strength.

To the first strong woman in my life my mother, Rebeh. Your sacrifices, your silent strength, and your constant love have carried me through it all.

To my beloved siblings Mohamed. Adam, Asmaa, Sorour, and Chahd thank you for being my joy, my support, and my safe place.

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And FINALLY, to myself I'm proud of the woman I've become. I've faced every challenge with strength, wiped my own tears, and stood tall. This achievement is proof that I never gave up and I never will.

GHADA

Dedication

To all those who hold a special place in my heart those to whom I owe every step of this journey:

To Allah, the Most Merciful, who granted me the strength, faith, and answered prayers without You, none of this would have been possible.

To the man who once called me the apple of his eye, who sees in my smallest achievement his greatest pride my father, ALI. Your support and love are my strength.

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To Salma Farhi, my supervisor thank you for your trust

And FINALLY, I dedicate this work to myself.

To the countless hours of effort, the persistence through challenges, and the unwavering belief that growth is born from struggle.
This journey has been a testament to my resilience, passion, and determination to achieve what I once thought impossible.

BASSET

Abstract

Anthocyanins are natural pigments with valuable functional properties, widely utilized as colorants in the food and pharmaceutical industries. Red onion **(Allium cepa L.)** peels, often treated as agricultural waste, are increasingly recognized for their richness in cyanidin-based anthocyanins and their potential as a sustainable source of natural dyes.

This study aimed to optimize anthocyanin extraction from red onion peels by investigating the effects of solvent acidity and extraction time. Two acidified ethanol solutions (70% v/v) were prepared using 0.01 M and 0.04 M HCl, and applied to dried peel powder (5 g) following a 20-minute maceration step at room temperature and incubation in a water bath at 30°C for 15 and 40 minutes. Extracts were filtered and analyzed spectrophotometrically using the pH differential method, with absorbance recorded at 520 nm and corrected at 700 nm.

The results showed that both acidity and extraction time had a significant positive effect on anthocyanin yield. The highest content, 581.57 mg/100g dry matter, was obtained with the solvent containing 0.04 M HCl after 40 minutes of incubation. Regression analysis confirmed the statistical significance of both factors and their interaction ($p < 0.001$), with an excellent model fit ($R^2 = 0.9984$), supporting the reliability of the findings.

These results demonstrate the efficiency of using acidified ethanol under mild thermal conditions for the extraction of anthocyanins from onion peels, offering a simple and eco-friendly alternative for natural pigment recovery.

Keywords: Anthocyanins, Red onion peels, Optimized extraction, Solvent acidity, Incubation time, Linear regression, Yield of natural pigments.

Résumé

Les anthocyanes sont des pigments naturels dotés de propriétés fonctionnelles précieuses, largement utilisés comme colorants dans les industries agroalimentaire et pharmaceutique. Les pelures d'oignon rouge (Allium cepa L.), souvent considérées comme des déchets agricoles, sont de plus en plus reconnues pour leur richesse en anthocyanes à base de cyanidine et leur potentiel en tant que source durable de colorants naturels.

Cette étude visait à optimiser l'extraction des anthocyanes à partir de pelures d'oignon rouge en étudiant l'effet de l'acidité du solvant et du temps d'extraction. Deux solutions d'éthanol acidifié à 70 % (v/v) ont été préparées en utilisant 0,01 M et 0,04 M de HCl, puis appliquées à 5 g de poudre de pelures séchées après une macération initiale de 20 minutes à température ambiante et une incubation au bain-marie à 30°C pendant 15 et 40 minutes. Les extraits ont été filtrés et analysés par spectrophotométrie selon la méthode différentielle de pH, avec des lectures à 520 nm corrigées à 700 nm.

Les résultats ont montré que l'acidité du solvant ainsi que le temps d'extraction ont eu un effet significatif et positif sur le rendement en anthocyanes. La teneur la plus élevée, soit 581,57 mg/100 g de matière sèche, a été obtenue avec le solvant contenant 0,04 M de HCl après 40 minutes d'incubation. L'analyse de régression a confirmé la signification statistique des deux facteurs et de leur interaction ($p < 0,001$), avec un excellent ajustement du modèle ($R^2 = 0,9984$), confirmant la fiabilité des résultats.

Ces résultats démontrent l'efficacité de l'utilisation de l'éthanol acidifié sous des conditions thermiques modérées pour l'extraction des anthocyanes à partir de pelures d'oignon, offrant ainsi une alternative simple et écologique pour la récupération des pigments naturels.

Mots-clés : Anthocyanines, Pelures d'oignon rouge, Extraction optimisée, Acidité du solvant, Temps d'incubation, Régression linéaire, Rendement en pigments naturels.

ملخص

الأنثوسيانينات هي أصباغ طبيعية تتمتع بخصائص وظيفية قيّمة، وتُستخدم على نطاق واسع كملونات في الصناعات الغذائية والدوائية. تُعد قشور البصل الأحمر(.Allium cepa L) ، التي تُعتبر غالبًا من النفايات الزراعية، مصدرًا غنيًا بالأنثوسيانينات من نوع السيانيدين، وتُكتسب اهتمامًا متزايدًا كمصدر مستدام للأصباغ الطبيعية.

هدفت هذه الدراسة إلى تحسين استخلاص الأنثوسيانين من قشور البصل الأحمر من خلال دراسة تأثير حموضة المذيب ومدة الاستخلاص. تم تحضير محلولين من الإيثانول بتركيز 70 (v/v) %محمّضين بحمض الهيدروكلوريك بتركيزين مختلفين (0.01 مول/لتر و0.04 مول/لتر)، وطُبّقا على مسحوق القشور المجففة (5 غرام) بعد نقع مبدئي لمدة 20 دقيقة في درجة حرارة الغرفة، تلاه حضن في حمام مائي بدرجة حرارة 30 °C لمدة 15 و40 دقيقة. بعد الترشيح، تم تحليل المستخلصات طيفيًا باستخدام طريقة الفرق في الأس الهيدروجيني، مع تسجيل الامتصاصية عند 520 نانومتر وتصحيحها عند 700 نانومتر.

أظهرت النتائج أن لكل من الحموضة ومدة الاستخلاص تأثيرًا إيجابيًا وملحوظًا على مردودية الأنثوسيانين. وقد سُجل أعلى تركيز (581.57 ملغ/100غ من المادة الجافة) باستخدام المذيب المحتوي على 0.04 مول/لتر من HCl بعد 40 دقيقة من الحضن. أكدت تحاليل الانحدار الأهمية الإحصائية لكل من العاملين وتفاعلهما($p < 0.001$) ، مع توافق ممتاز للنموذج($R^2 = 0.9984$) ، مما يعزز مصداقية النتائج.

تُظهر هذه النتائج فعالية استخدام الإيثانول الحمضي تحت ظروف حرارية معتدلة لاستخلاص الأنثوسيانينات من قشور البصل، مما يوفر بديلًا بسيطًا وصديقًا للبيئة لاسترجاع الأصباغ الطبيعية.

الكلمات المفتاحية: أنثوسيانينات، قشور البصل الأحمر، استخلاص محسَّن، حموضة المذيب، مدة الحضانة، الانحدار الخطي، مردود الأصباغ الطبيعية.

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List of abbreviations

Abbreviation	Full Term
%	Percentage
°C	Degree Celsius
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of Variance
AOX	Antioxidant
BBD	Box-Behnken Design
C3,5dG	Cyanidin-3,5-diglucoside
C3G	Cyanidin-3-glucoside
C6-C3-C6	Basic flavonoid structure: two aromatic rings (C6) linked by a three-carbon chain (C3)
CCD	Central Composite Design
CO_2	Carbon dioxide
DESs	Deep Eutectic Solvents
DoE	Design of Experiments
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Ea	Activation energy
EAE	Enzyme-Assisted Extraction
EtOH	Ethanol
FDA	Food and Drug Administration
FOS	Fructooligosaccharides
GAE	Gallic Acid Equivalent
GRAS	Generally Recognized as Safe
HCl	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
k	Rate constant
MAE	Microwave-Assisted Extraction
MeOH	Methanol
mg/g	Milligrams per gram
mg/g DW	Milligrams per gram of dry weight
NADES	Natural Deep Eutectic Solvents
OVAT	One-Variable-At-a-Time
pH	Potential of Hydrogen
R^2	Coefficient of Determination
ROS	Reactive Oxygen Species
RSM	Response Surface Methodology
S-alk(en)yl	Sulfur-alk(en)yl group

SFE	Supercritical Fluid Extraction
TPC	Total Phenolic Content
UAE	Ultrasound-Assisted Extraction
UV	Ultraviolet
w/v	Weight/Volume ratio

General introduction

Natural colorants represent a promising alternative to synthetic dyes, which are often criticized for their potential harmful effects on human health and the environment. Among these natural pigments, anthocyanins hold a significant place due to their antioxidant, anti-inflammatory properties and their wide color range from red to blue **(He & Giusti, 2020).** These water-soluble compounds, classified as flavonoids, naturally occur in a variety of fruits, vegetables, and flowers.

Onion peels, commonly regarded as agro-industrial waste, are now recognized as a rich source of anthocyanins, especially in red and purple onion varieties **(Cheng et al., 2022).** The valorization of these by-products aligns with circular economy principles and waste reduction strategies, while offering a sustainable solution for obtaining high-value natural colorants.

However, anthocyanin extraction is a complex process influenced by various factors such as temperature, pH, solvent type, and extraction time. Moreover, the stability of anthocyanins is highly dependent on environmental conditions. In this context, optimizing extraction conditions is crucial to improve yield and preserve the quality of the extracted compounds **(Tena & Asuero, 2023).**

The main objective of this study is to optimize the extraction of natural anthocyanins from onion peels using modern and environmentally friendly techniques. The work relies on statistical modelling to identify optimal extraction conditions, while exploring innovative methods such as ultrasound-assisted and microwave-assisted extraction, as well as the use of green solvents.

This thesis is structured into three main parts:

- A **literature review** that presents the properties of anthocyanins, their natural sources, and highlights the potential of onion peels.
- An **experimental section** describing the applied extraction protocols and the variables tested for optimization.
- A **results and discussion section**, where the findings are analyzed and compared with recent literature to validate the effectiveness of the proposed conditions.

Bibliographic part

Chapter I: Anthocyanins

Introduction to Anthocyanins

Anthocyanins are a group of water-soluble pigments belonging to the flavonoid family, and they are responsible for the strong red, purple, and blue colors in many fruits, vegetables, and flowers. They are secondary plant metabolites synthesized by the phenylpropanoid pathway, and their presence not only contributes to the attractiveness of plants but also many physiological processes such as protection against UV, pollinator attraction, and defence against pathogens **(Khoo et al., 2022).**

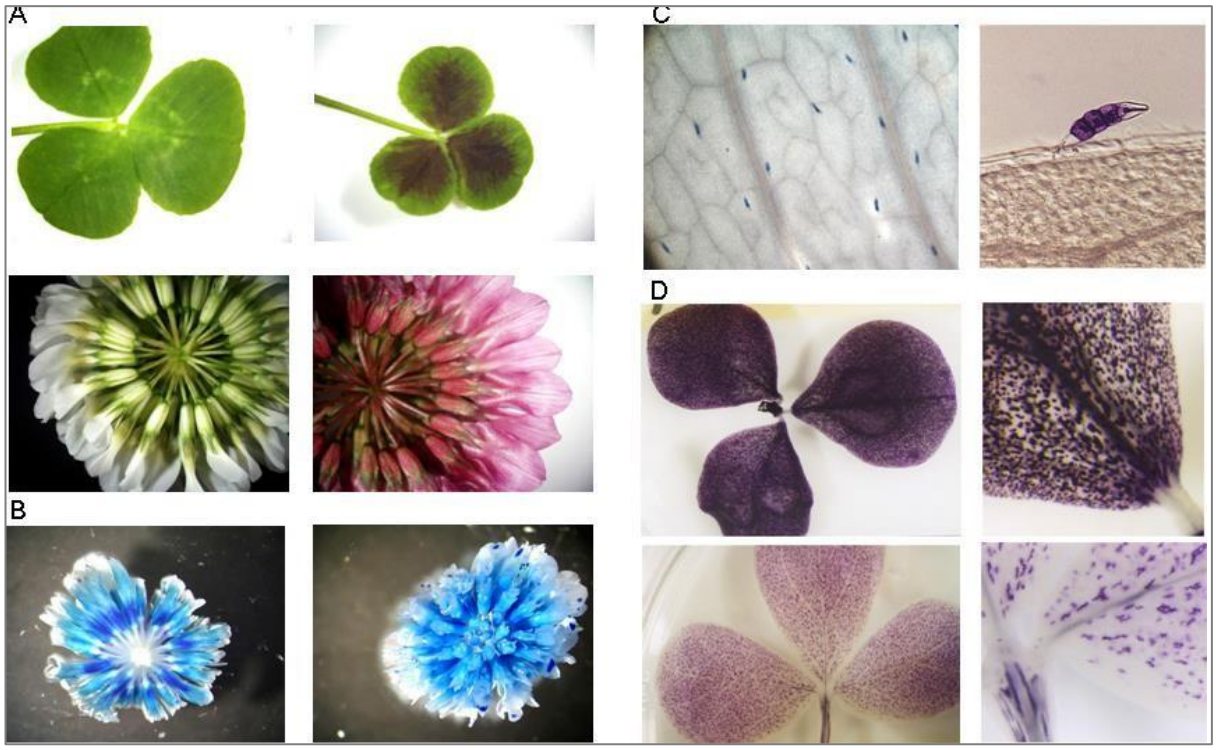


Figure 1: The different colorations produced by the presence of anthocyanins (Abrahams et *al.*, ,2002)

The growing scientific interest in anthocyanins is their multifunctionality. Aside from their function in plants, they have also been of extreme interest for their possible health-promoting effects in humans, such as antioxidant, anti-inflammatory, and anti-carcinogenic activities. This has spawned a multitude of studies aimed at the extraction of anthocyanins from natural sources, and especially from agro-industrial wastes like onion peels, which contain high amounts of these valuable compounds **(Rodriguez-Amaya, 2021).**

The research on anthocyanins as natural and bioactive colorants is within the realm of demand for clean-label and sustainable food and nutraceutical ingredients. With synthetic colorants being increasingly scrutinized, anthocyanins offer a potential candidate with an acceptable balance of effectiveness, safety, and eco-friendliness **(Giusti & Wrolstad, 2023).**

1 History of anthocyanin

Anthocyanins are pigments found in plants that belong to the flavonoid group of compounds and are responsible for the red, blue, and purple colors of plants. The term "anthocyanin" was given in 1835 by the German physicist Ludwig Clomer Marquardt in his publication Die Farben der Blüthen (Romantic Colors), in which he shared this name with blue pigments in 1913, the German Richard Willstätter isolated anthocyanins from corn petals, resulting in knowledge of their precise structure. Willstätter received the Nobel Prize in Chemistry in 1915 for his work on plant pigments **(Smith et al., 2021).**

The first crystalline anthocyanin, bromine, was isolated from Primula polyanthaeum owners by British actress Rose Scott-Moncrieff in 1930. The credit is not just because of its amazing crystalline anthocyanin discovery,which has played a significant role in the development of plant pigment biochemistry knowledge **(Patel et al., 2021; Zhao et al., 2022).**

1.2 Nature and Importance

Anthocyanins are a water-soluble class of flavonoid pigments that are located in the vacuoles of cells in higher plants. They belong to a ubiquitous group of compounds called the polyphenolics,
and are the cause of the extensive range of colours of fruits, vegetables, and flowers. Besides, anthocyanins are its glycosylated forms which were named from anthocyanidin, and are very sensitive to pH, temperature, light, enzymes, or metal ions **(Wang et al., 2020).**

1.3 Anthocyanins' composition

Anthocyanins are water-soluble pigments that fall under the flavonoid subclass of polyphenols. Anthocyanins, chemically, are anthocyanidin glycosides—aglycone forms that represent the fundamental chromophores.

The fundamental form of anthocyanidins is the flavylium cation (2-phenyl benzo Pyridiu), and variations between them exist in terms of the number and location of hydroxyl and methoxyl substituents at the B-ring **(Heet al., 2022).** The most widespread anthocyanidins are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin. These compounds are usually conjugated with sugar moieties like glucose, rhamnose, galactose, which increase their stability and solubility **(Kang et al., 2021).** Besides glycosylation, anthocyanins are also modified by acylation with aliphatic or aromatic acids, which are responsible for enhanced pigmen stability and color intensity, especially in fluctuating pH and temperature **(Zhao et al., 2023).** The structure of anthocyanins significantly affects their bioactivity, color expression, and chemical behavior in food and pharmaceutical products. Anthocyanin diversity also depends on plant species, developmental stage, and environmental conditions like light and temperature **(Martins et al., 2020).**

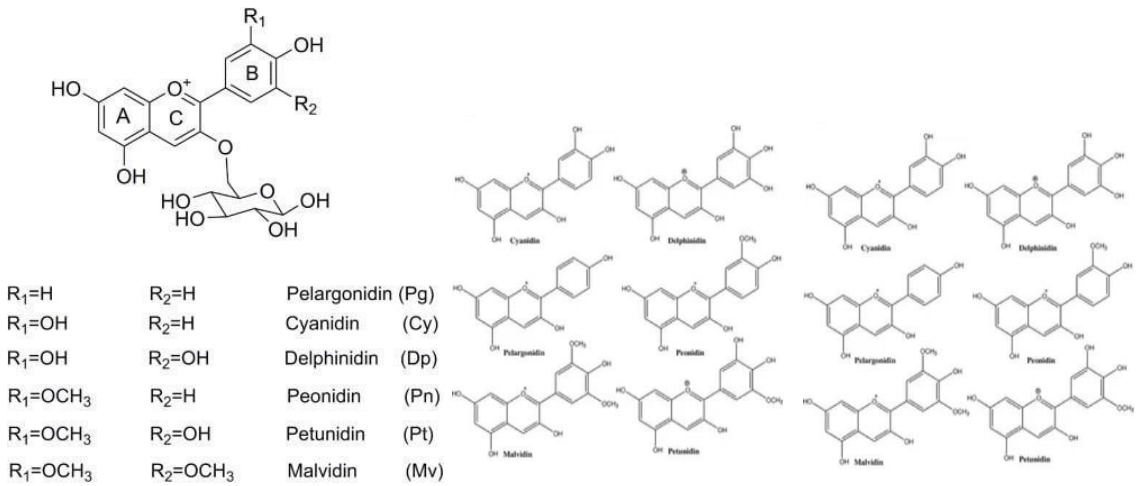


Figure 2: basic structure and different types of anthocyanins (Martins, N.et al 2020)

2. Anthocyanin Structure and Function

2.1 Chemical Structure of Anthocyanins

Anthocyanins are water-soluble flavonoid compounds that are characterized by a basic aglycone structure, called anthocyanidin, which is linked to sugar moieties (glycosides) in the plant. The fundamental structure consists of a flavonoid backbone, consisting of two aromatic rings (A and B) connected by a three-carbon chain, creating a structure known as C6-C3-C6. The structure's central feature is the presence of a positively charged oxygen in the C-ring, which is responsible for the pigment's color, particularly its red, purple, and blue hues. The color of anthocyanins can vary depending on the pH, which affects the stability and absorption characteristics of the molecule. This structure is influenced by the type of sugar (e.g., glucose, rhamnose) attached to the anthocyanidin, as well as any hydroxyl or methoxy groups present on the aromatic rings **(Gao et al., 2021).**

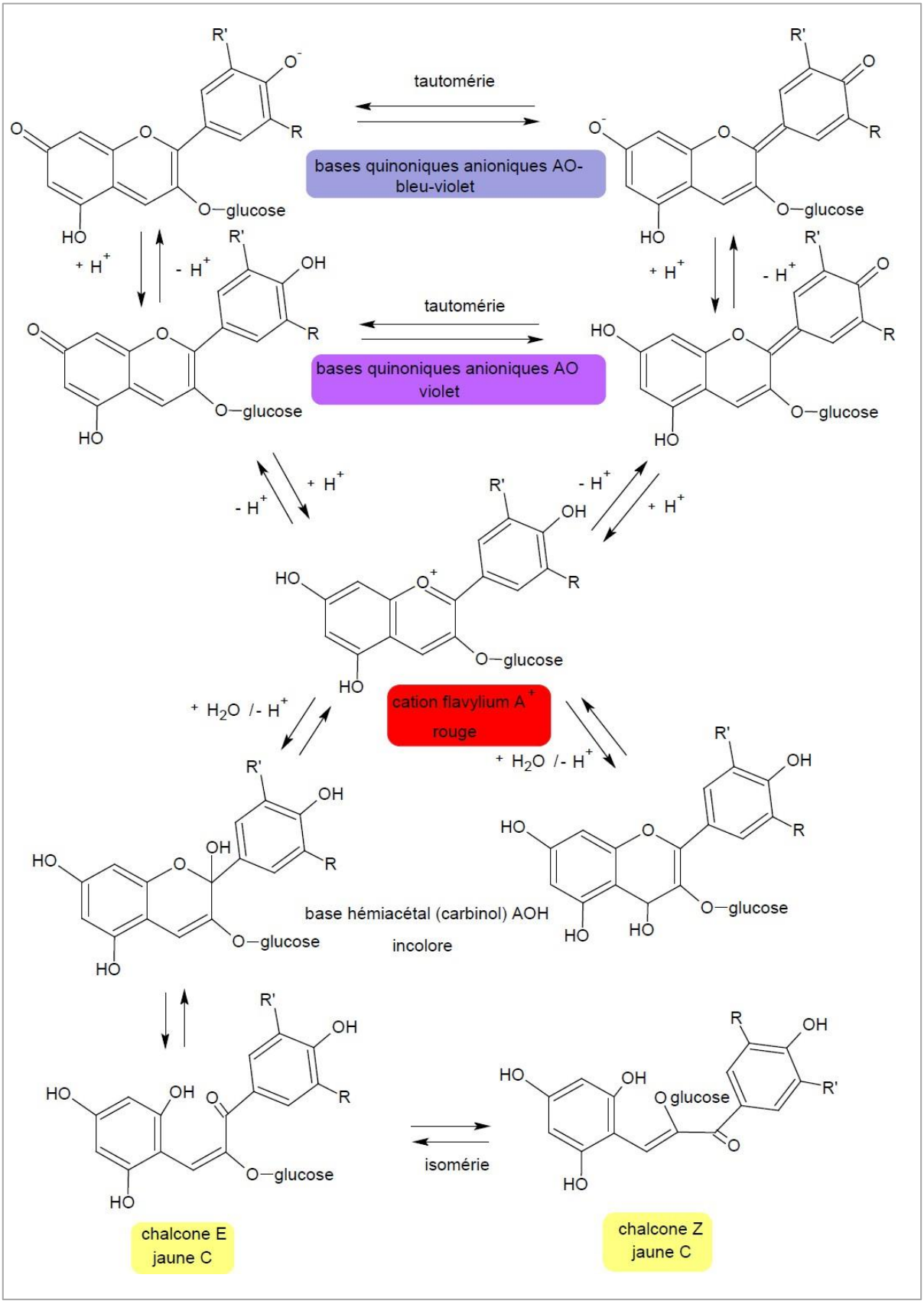


Figure 3 : Structural transformations of anthocyanine in solution (Brouillard et Lang, 1990).

2.2 Variation in Anthocyanin Structures

There are over 30 different anthocyanins identified in nature, and their structure can vary in terms of the attached sugar residues, the hydroxylation pattern on the aromatic rings, and the presence of additional modifications like acylation. These structural variations contribute to the diversity in their absorption spectra, stability, and reactivity, which are critical for their functionality both in plants and for human health. For instance, the presence of additional sugar groups can enhance solubility in water, thus affecting their bioavailability **(Lee et al., 2023).** Studies also indicate that acylated anthocyanins, which have additional acyl groups attached to their sugar moiety, tend to have more stable color characteristics and can be more resistant to degradation by light and heat **(Huang et al., 2022).**

3. Sources of Anthocyanins

3.1 Natural Sources of Anthocyanins: Fruits, Vegetables, and Flowers Fruits

Fruits are among the richest sources of anthocyanins. **Berries**, such as **blueberries, blackberries, elderberries, raspberries, strawberries**, and **chokeberries (Aronia)**, contain significant concentrations of anthocyanins, particularly cyanidin, delphinidin, and malvidin derivatives. For example, **blueberries** are rich in malvidin-3-glucoside and delphinidin-3-galactoside, whereas **black raspberries** contain high levels of cyanidin-3-glucoside **(Pojer et al., 2020; Kim et al., 2022).** Other anthocyanin-containing fruits include **red grapes**, **plums**, **pomegranates**, and **cherries**, each characterized by a specific anthocyanin profile that depends on species, cultivar, and ripeness **(Zhao et *al., 2021*).**

Vegetables

Several vegetables are valuable sources of anthocyanins. Notably, **red onions**, **purple sweet potatoes**, **eggplants**, **red cabbage**, and **purple corn** contain high concentrations of cyanidin, delphinidin, and peonidin derivatives. In **red onions**, anthocyanins are concentrated in the **outer peels**, with cyanidin-3-glucoside being the major compound. **Purple sweet potatoes** contain stable acylated anthocyanins like peonidin-3-caffeoyl sophoroside-5-glucoside, which are notable for their enhanced thermal and pH stability **(Yeh et al., 2023; Zhao et al., 2021).**

Flowers

Anthocyanins are also abundant in the petals of many edible and ornamental flowers. **Hibiscus sabdariffa**, **Clitoria ternatea (butterfly pea)**, **roses**, and **violas** are common floral sources. For example, **hibiscus extracts** are rich in delphinidin and cyanidin glycosides and are used in food and beverages for both coloring and antioxidant functionality **(Ali et al., 2021). Clitoria ternatea** provides vivid blue anthocyanins known as **ternatins**, which show high color stability and have gained popularity in natural food coloring applications **(Li et al., 2024)**

3.2 Function in Plants

In plants, anthocyanins are primarily involved in the protection against various environmental stressors. Their antioxidant properties allow them to neutralize harmful reactive oxygen species (ROS), which can damage plant cells due to exposure to UV radiation and other environmental stresses. Anthocyanins also play a key role in protecting plant tissues from oxidative damage during freezing, drought, and high-light stress **(Li et al., 2020).** Furthermore, anthocyanins are involved in attracting pollinators and seed dispersers through their vibrant colors, which is essential for the plant's reproductive success **(Wang et al., 2022).**

4 Common Onion kinds and Anthocyanin Content

There are several commercially important types of onions with varying parcels red onions have anthocyanins, primarily cyanidin-3-glucoside and its derivations. These colors give the onions red to grandiloquent color. They're mild to pungent in taste. unheroic onions are unheroic to brown in color on the outside and have a strong, pungent flavor. They contain smaller anthocyanins but are rich in quercetin. White onions warrant anthocyanins and are sweet and mild in taste. White onions are lower in phenolic and flavonoid composites. Sweet onions, maybe pale unheroic or white, have minimum pungency with lower sulfur content. Sweet onions vary in anthocyanin content with cultivars. Shallots are sanguine- pink on the surface. They retain less robust flavors and bear a moderate quantum of anthocyanins and flavonoids **(Nguyen et al., 2021)**



Figure 4: Close-up view of sliced red onion rings showing the vibrant anthocyanin-rich layers.

Source: Image by Freepik (Accessed May 22, 2025)

4.1 Relevance of Onion Peel as an Agro-Industrial Waste with High Anthocyanin Content Onion peels, a major by-product of the onion processing industry, are generated in vast quantities during cleaning, trimming, and slicing operations. Traditionally discarded as waste, these peels have recently gained scientific and industrial attention due to their **rich phytochemical profile**, particularly their high content of **anthocyanins**, flavonoids, and other polyphenols. As the global demand for sustainable food systems increases, valorizing such underutilized agricultural residues offers both environmental and economic advantages. **(Teng et al., 2021).**

Among the varieties of onions, **red and purple cultivars** are particularly notable for their high anthocyanin concentrations, with cyanidin-3-glucoside being the predominant pigment **(Mogren et al., 2020).** These pigments are mainly concentrated in the outermost dry layers of the bulb, making the peels an excellent and **cost-effective source** of natural colorants and antioxidants **(Sharma et al., 2022).**

Utilizing onion peels as a **functional raw material** not only supports the principles of **circular bioeconomy** and waste reduction but also aligns with clean-label trends and the push for plant-based ingredients in food, pharmaceutical, and cosmetic formulations **(Zhu et al., 2024).** Moreover, several studies have highlighted their anti-**inflammatory,**

antimicrobial, and anticancer potential, further reinforcing their value beyond coloration **(Figueroa et al., 2023).**

Given their availability, low cost, and high anthocyanin yield, onion peels are increasingly being explored for **green extraction processes** aimed at maximizing recovery while minimizing environmental impact **(Gupta et al., 2022).**

5 Chemical Composition of Onion Types

Onion types vary in their chemical composition. humidity content generally ranges from 88 to 92, varied by type. The content of anthocyanin is loftiest in red onions in the range 0.1- 1.5 mg/ g fresh weight, while the content is null or negligible in white onions. Fiber content in onions is 1.0 to 2.5 grams per 100 grams of fresh weight, which is the reason for their prebiotic exertion. Sugar content is 3 to 6 grams, impacting the agreeableness and overall flavor. pH values are slightly acidic, ranging from 5.3 to 6.2. Sulfur- containing composites similar as thiosulfates and sulfoxides are the reason for pungency as well as contribute to antioxidant exertion **(Cheng et al., 2021).**

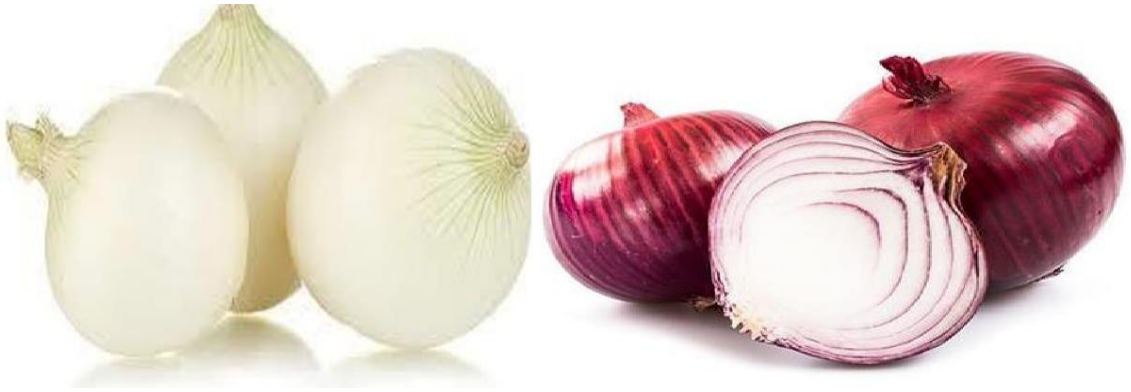


Figure 5: **white and red onion: Image by Freepik (Accessed march 11, 2024).**

5.1 Botanical and Chemical Composition of Various Types of Onion

Onions (Allium cepa L) are extensively grown and used globally because of their flavor and culinary applications. Onions are mainly classified into three main categories by color: white, yellow, and red. red onions among them have been of the greatest interest as a source of anthocyanins because they are known to contain high levels of these water-soluble pigments in the outer peel tissues **(Ghasemi et al., 2021; Griffiths et al., 2022).**

in anthocyanins, mainly in the form of glycosides of cyanidin. Quercetin and kaempferol are other phenolic compounds found therein, which are responsible for onion peel's antioxidant activity **(Naczk et al., 2020).** Other than these phytochemicals, onion peels also contain dietary fiber, which is responsible for digestive health maintenance **(Bourgeois et al., 2021)**

Table 1: Anthocyanin Content and Major Anthocyanin Compounds in Different Onion Peel Types

5.2 Anthocyanin Content in Red Onion Peels

Table 1: – Anthocyanin Content and Major Anthocyanin Compounds in Different

Onion Type	**Anthocyanin Content (mg/100 g dry weight)**	**Major Anthocyanin Compounds**
Red Onion Peels	210–350	Cyanidin-3-glucoside, Cyanidin-3-lactoside
Yellow Onion Peels	5–20	Trace cyanidin derivatives
White Onion Peels	<1 or not detected	Not detectable

Onion peel types:

red onion peels are particularly rich in cyanidin derivatives, such as cyanidin-3-glucoside, which is considered the predominant anthocyanin in this matrix (López-Martínez et al., 2020). The concentration of anthocyanins in peels is significantly higher than in the edible bulb portion, due to the plant's defenses mechanisms and the role of pigments in protecting the outer tissues from UV radiation and microbial invasion **(Rico et al., 2021).** Moreover, the anthocyanins found in onion peels often exist in acylated forms, enhancing their color stability and making them attractive for use as natural colorants in food systems **(Gani et al., 2023).**

5.3. Importance and Applications

5.3.1 IMPORTANCE OF NATURAL PIGMENTS IN FOOD:

Natural pigments, especially those derived from plant sources such as fruits, vegetables, and flowers, play a significant role in the food industry. Beyond their primary function of imparting appealing colors to food products, these compounds contribute to the nutritional and functional quality of foods. Among the most studied natural pigments are anthocyanins, carotenoids, chlorophylls, and betaines, which are not only safe and biodegradable but also exhibit strong antioxidant, anti-inflammatory, and antimicrobial properties **(Kaur et al., 2021; Gómez-Maqueo et al., 2023).**

The increasing consumer demand for clean-label products and growing concerns over the toxicity and long-term health effects of synthetic dyes have accelerated the shift towards natural food colorants. Natural pigments enhance product appeal and consumer perception of healthiness, making them valuable ingredients in functional foods, nutraceuticals, and beverages Furthermore, these compounds often serve dual roles, acting as both colorants and bioactive agents, thus contributing to chronic disease prevention through mechanisms related to oxidative stress reduction and immune modulation. **(Martins et al., 2022).**

In cosmetics

natural pigments are incorporated into lipsticks, foundations, and skincare products. Their appeal lies in both their vibrant colors and their skin-beneficial properties. For example, flavonoid-based pigments have been found to reduce oxidative stress on the skin and protect against UV-induced damage, making them suitable for natural sunscreens and anti-aging Moreover, consumer preference for plant-based, non-toxic ingredients reinforces their adoption in cosmetic formulations. formulations **(Alfieri et al., 2020).**

In the Pharmaceutical Industry

Natural pigments are also increasingly used in pharmaceutical formulations, not only for visual identification but also for their therapeutic potential. Compounds like curcumin and anthocyanins have shown promising pharmacological effects, including anticancer and neuroprotective activities Their bioactive nature enables them to serve as functional ingredients in nutraceuticals and drug delivery systems. **(Tan et al., 2021).**

6 Increase in Demand for Plant-Based, Clean-Label Colorants

In the last several years, consumer health consciousness about food safety, sustainability, and general well-being has grown exponentially. This has backed a worldwide trend for clean-label foods, where consumers are intentionally looking for foods and drinks produced using natural, minimally processed, and familiar ingredients **(Aschermann-Witzel et al., 2021).**

Artificial colorants—traditionally linked with possible health dangers and regulatory strain—are being substituted with plant-based colorants, most conspicuously those extracted from vegetables and fruits. Of these, anthocyanins—water-soluble flavonoid pigments that are responsible for red, purple, and blue colors in numerous plant tissues—are being progressively placed in the spotlight because of their dual role as colorants and bioactive molecules, Demand for eco-friendly and health-benefiting substitutes has also fueled the investigation of agro-industrial by-products, e.g., onion peels, as low-cost and environment-friendly sources of anthocyanins **(González-Barrio et al., 2021; Sani et al., 2023).** This is based on circular economy concepts and the recent drive towards waste valorization and green extraction technologies.

Recent developments have aimed at the simplification of extraction procedures to reach industrial scale, increased pigment yields, and better stability of anthocyanins under different processing conditions **(Li et al., 2023; Kumar et al., 2022).**

7 Functional Foods and Nutraceuticals Applications

Anthocyanins have received considerable attention recently for their application in nutraceuticals and functional foods since they exhibit health-beneficial bioactivities and natural colorant properties. In addition to aesthetic improvement of a product, these pigments play a role in the prevention of chronic diseases, aligning with consumers' expectations for clean-label and health-oriented products **(Khoo et al., 2020; Ntagkas et al., 2021).**

Functional Foods

Functional foods are foods that provide health value in addition to fundamental nutrition. Anthocyanins are becoming more and more added to drinks, yogurts, cereals, snacks, and meat substitutes. For instance, extracts of purple corn, black rice, and berry pomace containing anthocyanins are utilized to enrich yogurts, smoothies, and bakery products. These ingredients enhance not just the antioxidant potential of the food matrix but

also, its commercial value as a "natural" and "health-promoting" product **(Petroni et al., 2020; Urquiaga et al., 2023).**

Anthocyanins were also investigated for their role as modulators of gut microbiota activity, where they have a prebiotic effect upon their application in fermented cereal and dairy foods. Their impact on intestinal flora is beneficial to intestinal well-being, immune system modulation, and metabolic control, which makes them worthwhile to utilize in probiotic-enriched functional foods **(Ravi et al., 2022).**

8 Anthocyanins as Functional Ingredients in Foods

Anthocyanins are being more and more investigated for use as functional food ingredients because of their natural antioxidant activity, inhibition of food spoilage, and suggested health-promoting properties. As natural colorants, anthocyanins are favored over synthetic food colorants in foods and beverages because of their natural nature and health-enhancing properties. Apart from this, their inclusion in functional foods, including smoothies, dietary supplements, and fortified foods, has been taken seriously due to the potential they possess in enhancing public health **(Zhao et al., 2021).**

Apart from this, anthocyanin stability and bioavailability are crucial to their effectiveness, and processes like encapsulation and microencapsulation are applied to improve their stability in food processing and storage **(Huang et al., 2022).**

Health Benefits

Health Benefits of Anthocyanins: Anthocyanins have broad biological activities that have earned them increasing recognition as outstanding functional bioactive compounds. Among the most well-researched healthy-promoting activities are their antioxidant, anti-inflammatory, and anti-cancer activities, making them valuable therapeutic compounds in the prevention and treatment of chronic diseases **(Khoo et al., 2020; He et al., 2021).**

Anthocyanins are effective free radical scavengers that can neutralize reactive oxygen species (ROS) and inhibit the oxidative degradation of biomolecules like lipids, proteins, and DNA. They owe their antioxidant activity primarily to hydroxyl groups on their B-ring skeleton that donate hydrogen atoms to radicals for stabilization **(Pojer et al., 2021).** Experiments showed that anthocyanin extracts from berry, red onion, and black rice elevate in vivo redox status and minimize oxidative stress markers in animal models and human studies **(Zhao et al., 2023).**

Anthocyanins also regulate inflammatory responses through downregulating inflammatory cytokines TNF-α, IL-6, and IL-1β, and inhibiting key signaling cascades NF-κB and MAPK. This anti-inflammatory effect has been confirmed in models of colitis, arthritis, and metabolic syndrome, with decreases in tissue inflammation and systemic markers. The capacity of anthocyanins to interact with immune cells and endothelial cells positions them as strong candidates for nutritional interventions in inflammatory diseases. **(González-Barrio et al., 2022).**

Anthocyanins display anti-cancer properties by various mechanisms such as apoptosis induction, cell cycle inhibition, angiogenesis inhibition, and regulation of oncogenic signaling pathways **(Tang et al., 2021).** Anthocyanin extracts have been in vitro and in vivo proven to inhibit the growth of various cancer cell lines like colon, breast, prostate, and lung cancers. For instance, cyanidin-3-glucoside has shown strong cytotoxicity against human colorectal cancer cells via caspase-dependent apoptosis activation and mitochondrial damage **(Kumar et al., 2024).** Besides, anthocyanins have also been observed to improve endothelial function, lower blood pressure, and augment circulation, all of which are cardiovascular-friendly **(Gao et al., 2021).** Of particular note also are the neuroprotective properties of anthocyanins, with research determining that they are able to reduce the risk of neurodegenerative disease like Alzheimer's by decreasing oxidative stress in the brain and improving memory and cognitive function (**Wang et al., 2022).**

Chapter II: Extraction Techniques for Anthocyanins

1 Historical Background

Anthocyanins are a subclass of flavonoids, plant pigments of natural occurrence that provide bright red, purple, and blue colors to the majority of fruits, vegetables, and flowers. They have been of interest to researchers for several years due to their suggested health effects like antioxidant, anti-inflammatory, and anti-carcinogenic effects, as well as applications as natural food colorants and nutraceuticals **(Khoo et al., 2022).**

Conventional anthocyanin extraction relied on rudimentary procedures such as maceration and decoction using water or ethanol as major solvents. These primitive procedures were convenient but often unsuccessful, generating unstable yields and chances of degradation of anthocyanins due to extended heat and exposure to oxygen. **(Castañeda-Ovando et al., 2020)**

Recent publications highlight that while maceration remains at the centre, modern advancements have elevated temperature and pH control to mitigate degradation and enhance recovery **(Narang et al., 2022).** While traditional processes like maceration and decoction were easy to perform, they were usually inefficient and poorly reproducible, resulting in inconsistencies in anthocyanin yield and purity. During the mid-20th century, increasing alarm regarding the safety of man-made dyes and an increased interest in novel natural sources prompted the scientific community to develop more effective and standardized techniques for the extraction of anthocyanins. This action was a step toward mechanized processes aimed at optimizing yield and stability of the compounds **(Ma et al., 2021).** This period witnessed the transition from empirical to more controlled and scalable method **(Silva et al., 2021)**.

The last twenty years have witnessed significant advancements driven by the green chemistry principles and the requirement of sustainable non-toxic process. The development of advanced extraction processes such as ultrasound-assisted extraction **(UAE),** microwave- assisted extraction **(MAE),** supercritical fluid extraction **(SFE),** and pressurized liquid extraction **(PLE)** has revolutionized the recovery of anthocyanins from various plant sources. These processes not only increase yield extraction and reduce solvent use but also preserve the structural complexity and bioactivity of the anthocyanins **(Castañeda-Ovando et al., 2020; D'Urso et al., 2023).**

Moreover, the choice of extraction method has evolved parallel to improvements in analytical t technologies for better quantitation and characterization of anthocyanins. Today,
the choice of an efficient extraction process is influenced by factors including the raw material character, stability of the target compound, the environmental impact, as well as downstream processing **(Kumar et al., 2024).**

Generally, the evolutionary history of anthocyanin extraction reflects a broader shift in food and natural product chemistry—away from traditional, low-yielding processes and towards innovative, eco-friendly technologies. This shift has paved the way for optimum recovery of anthocyanin from agro-industrial residues, such as onion skins, based on the principles of circular economy and sustainable food systems **(Martins et al., 2021; Saini et al., 2022).**

Optimized extraction procedures are required for effective recovery of anthocyanins from plant materials, e.g., onion peels. The selection of an effective extraction technique has a direct consequence on the yield, purity, and stability of anthocyanins. With increasing consumer pressure for natural and clean-label products, researchers have strived increasingly to create new and more environmentally friendly extraction techniques with minimal solvents and lower environmental impact **(Ravichandran et al., 2020; Ma et al., 2021; Khoddami et al., 2020)**

2 Conventional Extraction Methods

Traditional solvent extraction has been the most prevalent method used to purify anthocyanin. Here, solvents, for example, ethanol, methanol, and acidified water, are used to remove the pigments under the best temperature and duration conditions. Albeit widely practiced due to ease of operation and cost-effectiveness, the procedure is not without shortcomings, for example, the need for lengthy extraction times, low yields, and the use of organic solvents with probable toxicity risks **(Khoo et al., 2021).**

2.1 Maceration

Maceration is one of the most traditional and straightforward techniques used for the recovery of anthocyanins from plant matrices. Maceration involves the immersion of the sample in an appropriate solvent—commonly aqueous methanol or ethanol acidified with a weak organic acid, such as citric or formic acid—under ambient or slightly elevated temperatures for a predetermined time, typically ranging from several hours to 24 hours **(Gonçalves et al., 2021).**

It is preferred because of its simplicity, low cost, and the minimal use of equipment. However, it is typically defined by long extraction times and lower extraction performance compared to advanced techniques. Maceration efficiency is affected by multiple factors including the solvent polarity, the solvent to solid ratio, temperature, and the time of extraction Despite the disadvantages associated with maceration, it is still prevalent among the initial screening of anthocyanin content of plant waste products such as onion peels **(Nadeem et al., 2023).**

2.2 Soxhlet Extraction

Soxhlet extraction is a conventional hot solvent extraction technique that is more efficient than maceration. It is based on the cumulative cycling of hot solvent through a thimble of solid plant material, enabling thorough extraction in repeated cycles. The technique is best suited for the extraction of thermally stable phenolic compounds with anthocyanins—since they are heat labile—potentially degrading over prolonged high-temperature use **(Park et al., 2022).**

The major advantages of Soxhlet extraction are automation, higher yield, and efficiency in solvent usage but also is limited by long extraction times (typically 4–8 hours), high energy requirements, and potential heat-induced degradation of heat-sensitive anthocyanins Therefore, it is more suited to preliminary estimation or where thermal degradation is minimal under mild conditions and thermostable solvents. **(Akhtar et al., 2024).**

3 Limitations of Conventional Extraction Methods: Time, Solvent Use, and Thermal Degradation

Although **maceration** and **Soxhlet extraction** are widely used for the recovery of anthocyanins from plant materials, these **conventional techniques** suffer from several inherent limitations that impact their efficiency, scalability, and environmental sustainability **(Silva et al., 2021; Tiwari et al., 2022).**

3.1 Long Extraction Time

Maceration requires extended contact between the solvent and the plant matrix, often ranging from several hours to overnight, which limits its industrial applicability. Similarly, Soxhlet extraction operates over continuous cycles that may last **4–8 hours or more**, consuming significant time, especially in batch processes **(Moghimi et al., 2023).**

3.2 Excessive Solvent Consumption

Both techniques typically involve **large volumes of organic solvents**, many of which are hazardous, flammable, or environmentally unfriendly. This leads to **increased operational costs**, challenges in **solvent recovery**, and concerns related to **residual solvents** in the final extract Acidified methanol and ethanol, although effective, still contribute to chemical waste and raise issues regarding **green chemistry compliance**. **(Maqsood et al., 2021).**

3.3 Risk of Thermal Degradation

Anthocyanins are **thermo-sensitive compounds** prone to degradation under high temperatures. In Soxhlet extraction, the constant heating of the solvent above 70 °C can cause **irreversible loss of pigment activity and antioxidant properties**, especially during prolonged operation. Even during maceration, if conducted at elevated temperatures to enhance diffusion, there remains a risk of partial degradation of anthocyanins. **(Park et al., 2022)**

4 Modern Green Extraction Techniques:

To overcome the drawbacks of conventional methods, modern green extraction techniques have emerged. These methods not only improve extraction efficiency but also align with principles of sustainability and eco-friendliness. Key techniques include:

• **Ultrasound-Assisted Extraction (UAE):** Uses ultrasonic waves to disrupt plant cell walls and enhance solvent penetration, reducing extraction time and increasing anthocyanin yield. UAE has been reported to improve recovery by 20–40% compared to conventional methods **(Nayak et al., 2022).**

• **Microwave-Assisted Extraction (MAE):** Applies microwave energy to heat solvents rapidly and uniformly, which facilitates the release of intracellular compounds. MAE significantly reduces extraction time (as short as 2–5 minutes) while maintaining anthocyanin integrity **(Mandal et al., 2020).**

• **Enzyme-Assisted Extraction (EAE):** Utilizes specific enzymes such as cellulase and pectinase to break down cell wall structures, improving anthocyanin release and reducing solvent requirements **(Wang et al., 2021).**

• **Supercritical Fluid Extraction (SFE):** Employs supercritical CO_2, often modified with co-solvents like ethanol, to selectively extract anthocyanins with high purity and minimal degradation. Though effective, SFE requires high equipment costs **(Zhang et al., 2022).**

5 Comparative Studies and Advantages of Modern Extraction Techniques

Over the past decade, **comparative studies** between traditional and modern extraction methods have consistently demonstrated the **superiority of green and advanced technologies** such as **ultrasound-assisted extraction (UAE)**, **microwave-assisted extraction (MAE)**, and **enzyme-assisted extraction (EAE)** for recovering anthocyanins from plant matrices, including onion peels **(Fattahi et al., 2022; Moghimi et al., 2023).**

5.1 Higher Yield and Efficiency

Modern extraction techniques have been shown to significantly **increase anthocyanin yield** due to enhanced cell wall disruption and better solvent penetration. For instance, UAE utilizes ultrasonic waves to create cavitation effects that **accelerate mass transfer** and **release intracellular compounds** more effectively than passive soaking or continuous reflux (**Bashmil et al., 2021).** MAE, on the other hand, causes rapid heating at the cellular level, promoting the release of anthocyanins in just a few minutes, compared to hours in Soxhlet or maceration **(Zou et al., 2020).**

5.2 Reduced Extraction Time

UAE and MAE drastically **reduce extraction time**—from hours to minutes—without compromising extract quality. UAE typically requires **5–30 minutes**, while MAE can extract anthocyanins in **less than 10 minutes** under optimized conditions, thereby increasing throughput and lowering energy consumption **(Ameer et al., 2022).**

5.3 Lower Solvent Usage and Environmental Impact

Green techniques are more **solvent-efficient** and aligned with sustainable practices. In comparative experiments, UAE and MAE consumed up to **50% less solvent** than traditional methods, supporting eco-friendly processing and facilitating compliance with green chemistry principles **(Tiwari & Brennan, 2022).**

5.4 Better Preservation of Bioactivity

Advanced methods often operate under **mild conditions** (UAE) or **controlled short bursts of heat** (MAE), which help preserve the **chemical integrity and antioxidant activity** of anthocyanins. These attributes are particularly important when anthocyanins are intended for **functional food or pharmaceutical applications (Maqsood et al., 2021).**

6 Factors Influencing Extraction:

Factors Affecting Anthocyanin Stability and Yield

The stability and extraction yield of anthocyanins are profoundly influenced by a variety of physicochemical and environmental factors, which must be carefully optimized to ensure maximum retention of their bioactivity during processing, storage, and application. These factors include pH, temperature, light exposure, oxygen availability, time, and the interaction with other matrix compounds such as metals and sugars **(Giuffrè et al., 2021; Castañeda-Ovando et al., 2022).**

6.1 pH Sensitivity

Anthocyanins are highly pH-sensitive molecules, existing in different chemical forms depending on the medium's acidity. In acidic environments (pH < 3), the red-colored flavylium cation form is dominant and stable. However, increasing the pH induces structural transformations into quinoidal bases (blue), carbinol pseudo bases (colorless), and chalcone forms (yellowish), resulting in color loss and degradation **(Bodart et al., 2023).**

6.2 Temperature and Thermal Degradation

Elevated temperatures can trigger hydrolysis of glycosidic bonds, oxidation, and the breakdown of the anthocyanin chromophore, especially during prolonged extraction or processing. Degradation typically follows first-order kinetics, with reaction rates increasing exponentially with temperature Therefore, lower-temperature, short-duration extraction methods such as UAE or cold maceration are preferred to preserve pigment integrity. **(Huang et al., 2020).**

6.3 Light and Oxygen Exposure

Anthocyanins are susceptible to photodegradation and oxidative reactions, especially in the presence of light and oxygen. Light induces cleavage of aromatic rings and loss of conjugated π-bonds, while oxygen can generate reactive oxygen species (ROS) that oxidize the chromophore Proper packaging (e.g., amber containers) and inert environments (e.g., nitrogen flushing) are essential for stability**. (Ali et al., 2021).**

6.4 Time of Exposure

Extended exposure to extraction conditions, particularly under high temperature or light, exacerbates anthocyanin degradation. Thus, optimizing extraction time is crucial for balancing yield and stability, especially in continuous processes **(Silva et al., 2023).**

6.5 Interaction with Other Compounds

Anthocyanins interact with metal ions (e.g., Fe^{3+}, Cu^{2+}), forming metal-anthocyanin complexes that may alter color and stability. These interactions can cause precipitation or color shifts. Additionally, co-existing sugars can stabilize anthocyanins through pigmentation or glycosylation, which enhances solubility and resistance to degradation **(Vasquez-Caicedo et al., 2024).**

7 Degradation Kinetics and Protection Strategies of Anthocyanins

Anthocyanins are inherently unstable compounds susceptible to chemical degradation, particularly under unfavorable environmental or processing conditions. Understanding their degradation kinetics provides valuable insights into optimizing formulation, processing, and storage conditions to preserve their stability and bioactivity (**He et al., 2021; Khoo et al., 2020).**

7.1 Degradation Kinetics

Anthocyanin degradation typically follows **first-order reaction kinetics**, where the rate of degradation is proportional to the concentration of the pigment. The degradation rate increases significantly with **temperature**, **pH elevation**, **light exposure**, and **oxygen availability**. The **half-life (t½)** of anthocyanins under given conditions is often used as a practical indicator of their stability. For instance, at higher temperatures (e.g., above 60°C), anthocyanins may show rapid decomposition, with their half-life reducing to a few hours or less (**Giusti & Wrolstad, 2020).**

7.2 Protection Strategies

To mitigate degradation and improve anthocyanin shelf life, several protection strategies have been developed:

- **Microencapsulation**: Entrapping anthocyanins in carriers such as maltodextrin, gum Arabic, or cyclodextrins protects them from environmental stressors. This technique enhances pigment stability during processing and storage, especially in food systems. **(López de Dicastillo et al., 2021; Fang & Bhandari, 2020).**
- **Use of Stabilizing Agents**: Co-pigmentation with compounds such as flavonoids, organic acids, or metal ions helps stabilize anthocyanin structures by forming molecular complexes, enhancing color intensity and resistance to degradation **(Bakowska-Barczak et al., 2020).**

- **Controlled pH and Storage Conditions**: Maintaining extracts in acidic pH (< 3.5), protecting them from light, and storing them at low temperatures under vacuum or inert atmospheres significantly reduces degradation rates **(Patras et al., 2022).**
- **Packaging Innovations**: Using UV-blocking films, oxygen-impermeable containers, or active packaging materials embedded with antioxidants can further protect anthocyanins from oxidative and photolytic degradation **(Nayak et al., 2021).**

These protection techniques are crucial for maintaining the functional and aesthetic properties of anthocyanin-containing products in food, nutraceutical, and cosmetic industries.

7.3 Influence of Extraction Conditions on Yield and Stability

The yield and stability of anthocyanins during extraction are highly dependent on several critical process parameters. Understanding the influence of these conditions is vital to optimize extraction protocols that maximize anthocyanin recovery while minimizing degradation **(Zhou et al., 2022).**

Solvent type and concentration are primary factors affecting extraction efficiency. Polar solvents such as ethanol and acidified water are preferred due to their ability to solubilize anthocyanins effectively. However, higher solvent concentrations do not always correlate with increased yield, as excessively high solvent polarity can lead to co-extraction of unwanted compounds, affecting purity and stability **(Kim & Lee, 2021).**

Temperature accelerates the diffusion of anthocyanins from the plant matrix, enhancing yield. Nonetheless, anthocyanins are thermolabile; exposure to high temperatures can result in degradation, manifested by reduced color intensity and antioxidant activity. Therefore, an optimum temperature range, typically between 30°C and 50°C, is recommended to balance yield and stability **(Pérez-Sánchez et al., 2023).**

pH plays a decisive role in anthocyanin stability. Under acidic conditions (pH 1–3), anthocyanins exist mainly as stable flavylium ions, preserving their color and antioxidant properties. At neutral or alkaline pH, anthocyanins rapidly degrade or transform into colorless forms, drastically reducing extract quality **(García et al., 2022).**

Extraction time and solid-to-liquid ratio also influence the process. Extended extraction times may improve yield but increase the risk of anthocyanin degradation due to prolonged exposure to oxygen and heat. Similarly, an optimal solid-to-liquid ratio ensures

sufficient solvent penetration for maximum extraction without unnecessary solvent waste or dilution effects **(Zhao & Wang, 2024).**

In conclusion, optimizing the interplay between key extraction parameters—such as solvent type, pH, temperature, extraction time, and solid-to-liquid ratio—is critical for maximizing anthocyanin yield while preserving structural stability. Such fine-tuned approaches are fundamental to ensuring the functionality of anthocyanins in food and pharmaceutical formulations, where both bioactivity and color integrity are essential **(Derrien et al., 2021).**

8 Optimization of Extraction

8.1 Experimental Design for birth Optimization

Experimental design methodologies play a basic part in the optimization of anthocyanin birth processes through the complete examination of the effect of various variables and their interactions. Of these methodologies, Response face Methodology (RSM) is greatly valued for its accomplishment in optimizing birth parameters like solvent concentration, temperature, pH, solid- to- liquid ratio, and birth time **(Singh et al., 2022).**

RSM facilitates the creation of intricate models describing the relationship between independent variables and the birth response, allowing the establishment of optimum conditions to realize maximum anthocyanin yield and stability **(Chen & Zhao, 2023).** RSM reduces experimental runs compared to traditional one- factor- at-a-time approaches, saving time and money while allowing deeper understanding of variable relationships **(Liang et al., 2021).**

The advisability function is usually incorporated in RSM to balance multiple response variables, for example, maximizing yield and maintaining anthocyanin integrity **(García et al., 2023).** Experimental design also enables scale- up considerations, freezing reproducibility and robustness of the optimized birth process in artificial operations **(Park & Kim, 2024).** In summary, the application of experimental design tools like RSM is needed for effective and orderly anthocyanin genesis optimization, leading to better process performance and product quality.

8.2 Role of Experimental Design

(e.g., Response Surface Methodology - RSM)

Experimental design techniques, in particular Response Surface Methodology (RSM), have proven to be essential tools for optimization of complex extraction processes such as anthocyanin extraction. RSM provides a systematic approach to screen a number of variables and their interactions at a time, enabling the identification of optimal extraction conditions using a minimum number of experimental runs compared to traditional one- factor-at-a-time methods **(Zhao et al., 2022).** By constructing predictive polynomial models, RSM enables the visualization of response surfaces and contour plots to understand how parameters like solvent concentration, temperature, pH, extraction time, and solid-to-liquid ratio influence the yield and quality of anthocyanins This approach not only maximizes extraction efficiency but also helps to minimize solvent consumption and thermal degradation. **(Wang & Liu, 2023).** Moreover, RSM enables multi-response optimization through desirability functions, balancing yield, purity, and stability of anthocyanin extracts **(Kumar et al., 2021)**. Stability and reproducibility of processes established through experimental design also promote scalability and industrial viability **(Singh & Verma, 2024).** In summary, the role of experimental design, more so RSM, in optimizing anthocyanin extraction processes is leading the way toward sustainable and efficient production

8.3 Key Variables Affecting Anthocyanin Extraction

The extraction efficiency and quality of anthocyanin are under the control of numerous interdependent variables, such as concentration and type of solvent, medium pH, temperature of extraction, time, and solid-liquid ratio. All the factors play a crucial role in influencing the rate of recovery as well as the chemical integrity of anthocyanins. It involves appropriate control and adjustment of these variables in order to get optimal yield as well as minimize degradation, particularly with the heat, pH, and oxidative sensitivity of anthocyanins **(Castañeda-Ovando et al., 2020).**

Solvent Concentration and Type: Solvent type has a bearing on the efficiency of extraction and solubility of anthocyanin. Acidified water, ethanol,
and methanol are typically used food-grade solvents. The optimal solvent concentration is

one that balances the polarity to extract anthocyanins effectively without breaking them **(García et al., 2022).**

pH: Anthocyanins are pH-sensitive, and pH changes influence their color stability and chemical conformation. Acid pH (pH 1–3)

generally, enhances anthocyanin stability and extraction yield by stabilizing the flavylium cation conformer **(Li et al., 2023).**

Temperature: Temperature improves rates of diffusion and

solubility, yet too high a temperature may lead to thermal degradation of anthocyanins. Ideal temperatures for extraction are generally 30–60°C **(Singh & Kumar, 2021).**

8.4 Extraction Time

Sufficient: time gives sufficient solubilization; but excess can result in degradation by oxidation or hydrolysis. Ideal time is therefore crucial **(Park et al., 2023).**

Solid-to-Liquid Ratio: The ratio affects concentration gradient driving mass transfer. Higher ratios increase extraction efficiency to a level of saturation beyond which there is no increment in yield **(Chen & Zhao, 2022).**

Optimizing these parameters collectively through experimental design maximizes the extraction yield and quality, as well as preserves the functional integrity of the anthocyanins for food and nutraceutical applications.

8.5 Statistical Modeling and Desirability Functions

Statistical modeling is key to optimizing anthocyanin extraction processes by precise analysis and forecasting of the impact of multiple variables on the yield and quality of extraction. Regression analysis, analysis of variance (ANOVA), and Response Surface Methodology (RSM) are common methodologies employed to develop mathematical models that specify the interaction between independent variables (e.g., solvent strength, pH, temperature) and response variables (e.g., anthocyanin yield, purity). Desirability functions add statistical modeling to guarantee a scientific approach to multi-response optimization. Each response is translated into a desirability value ranging from 0 (not desirable) to 1 (very desirable), which allows for the simultaneous optimization of multiple objectives such as maximizing yield, minimizing solvent usage or degradation, etc. **(Duan et al., 2022).**

The desirability function sums individual desirability's to one compound score that guides the identification of optimal process conditions that balance trade-offs between incompatible responses **(Singh & Verma, 2023).**

The approach enhances decision-making during process development, improving reproducibility and efficiency. Overall, statistical modeling combined with desirability functions is imperative for rational design and optimization of anthocyanin extraction to support scalable and sustainable production plans.

8.6 Optimization of Experimental Extraction of Anthocyanins

Optimization of anthocyanin extraction involves systematically adjusting process conditions to maximize yield, purity, and stability using less resources and damage. Experimental optimization typically employs design of experiments (DoE) methodologies, e.g., Response Surface Methodology (RSM), factorial designs, and central composite designs, to determine the effect and interaction of important variables like type and concentration of solvent, pH, temperature, extraction time, and solid-to-liquid ratio **(Patel et al., 2023).**

The scientists are able to find optimum extraction conditions that lead to maximum anthocyanin recovery with good reproducibility and efficiency through predictive model generation. It also lowers the requirement of solvents and energy, in line with green chemistry philosophy **(Alvarez et al., 2022).** Optimization studies tend to produce higher anthocyanin yield if the parameters of extraction are optimized, such as acidified ethanol at moderate temperatures and particular extraction times **(Rojas & Sánchez, 2024).**

Moreover, optimized extraction processes must be applied to preserve the bioactivity and functional characteristics of anthocyanins, which are important for their utilization in nutraceuticals, functional foods, and natural food colorants **(Chen et al., 2021).**

Experimental optimization of extraction parameters results in ongoing improvement, hence enhancing scalability and industrial relevance.

8.7 Solvent Considerations in Extraction

Solvent Considerations in Extraction Choice of solvent is an important element in the efficient extraction of anthocyanins because it directly affects yield, purity, and stability of compounds extracted. Ideal solvents need to possess relevant polarity to dissolve polar molecules that anthocyanins are usually assumed to be and, simultaneously, become safe for desired application, particularly in food and nutraceutical foodstuffs **(García et al., 2022).**

Ethanol, methanol, and acidified water are general solvents of which acidified water tends to be generally acidified by organic acids such as citric or formic acid to stabilize the anthocyanins through provision of an acidic environment that prevents degradation (Patel & Singh, 2023). Ethanol is widely used as it is a Generally Recognized as Safe (GRAS) solvent, non-toxic, and environment-friendly as per the principles of green chemistry **(Alvarez et al., 2021).**

Moreover, solvent concentration and use of mix solvent can optimize extraction efficiency through the balance of solubility and minimizing co-extraction of undesirable compounds **(Rojas & Sánchez, 2024).** Prudent selection of the solvent is also important to reduce environmental impact and ease downstream processing such as purification and recovery of the solvent.

Major factors to consider in the selection of the solvent for the extraction of anthocyanin include the enhancement of polarity, acidification, safety, environmental impact, and end use compatibility for high-quality extracts.

Preferential Use of Food-Grade and GRAS Solvents in Applications In the extraction of anthocyanins for use in foods, nutraceuticals, and pharmaceuticals, it is crucial that solvents that are food-grade and Generally Recognized as Safe (GRAS) are used to ensure consumer safety and adherence to regulatory requirements. Food-grade solvents such as ethanol and water are desirable due to their nontoxic nature and suitability for human consumption **(Kumar et al., 2023).**

The GRAS status, which is granted by government bodies like the FDA, indicates that such solvents are of limited health concern when used under defined conditions and are thus suitable for direct use in food-related processes without extensive purification (Singh & Patel, 2022). Additionally, these solvents facilitate green extraction approaches aligned with green chemistry principles, reducing environmental and workplace hazards **(Fernández et al., 2024).**

The use of food-grade and GRAS solvents not only enhances the safety profile of the anthocyanins to be extracted but also eases downstream processing, lowers production costs, and achieves greater consumer acceptance of the final products **(Wang et al., 2023).**

Their utilization is thus a key consideration in the formulation of efficient and safe anthocyanin extraction processes.

Solvent Selection Criteria for Solvent Selection The selection of an appropriate solvent for anthocyanin extraction is guided by some imperative factors to maximize the extraction efficiency, safety, and environmental sustainability. Some of the most significant ones include solvent polarity, safety, price, environmental impact, and compatibility with the intended application **(Rodríguez-Solana et al., 2022).**

Polarity is critical because anthocyanins are polar molecules; thus, solvents with moderate to high polarity such as ethanol, methanol, and acidified water tend to yield more extraction **(Zhang & Li, 2023).**

Safety considerations favor food-grade and GRAS solvents to ensure non-toxicity and regulatory approval of extracts for application as foods or therapeutics **(Kaur et al., 2024).**

Economic factors such as solvent cost, availability, and ease of recovery also influence selection, especially for large industrial-scale extractions. Environmental sustainability also encourages the use of green solvents with minimal toxic emissions and higher biodegradability **(Fernández et al., 2023).**

Lastly, solvent compatibility with downstream processing purification steps and anthocyanin stability during extraction must be considered to guarantee extract quality **(Patel & Singh, 2023).**

Common Solvents Used (Ethanol, Methanol, Acidified Water) Extraction of anthocyanins generally involves the use of solvents such as ethanol, methanol, and acidified water due to their ability to solubilize these polar pigments efficiently. Ethanol is the most preferred as it is food-grade, non-toxic, and environmentally friendly, and hence can be used in foods and pharmaceuticals **(Costa et al., 2023).**

Methanol, although highly efficient at anthocyanin extraction due to its high polarity, is toxic and primarily used in analytical or laboratory-scale extractions, not in foods **(Kumar & Singh, 2022).** Acidified water, usually adjusted with organic acids like citric or formic acid, stabilizes anthocyanins by the provision of a low pH environment that prevents degradation of the pigment during extraction **(Zhao et al., 2024).**

The choice among these solvents depends on the intended use, extraction yield, safety, and downstream processing needs. Mixing ethanol with acidified water is a common practice to achieve maximum anthocyanin yield while enhancing extract safety and stability **(Fernández et al., 2023).**

Chapter III: Onion as a Source of Anthocyanins

1 Introduction to Onion and Its Varieties

Onion (*Allium cepa L.*) is a globally cultivated vegetable belonging to the Amaryllidaceae family, widely recognized for its culinary uses and health-promoting phytochemicals. Among its varieties, red, yellow, and white onions are the most common, each differing notably in pigment composition, bioactive compounds, and nutritional properties. Red onions, in particular, are distinguished by their rich anthocyanin content concentrated mainly in their outer peels, making them a valuable natural source for pigment extraction. **(Carvajal et al., 2021).**

Table 2: Anthocyanin Content in Different Onion Varieties

Onion Type	Peel Color	Anthocyanin Content (mg/100 g DW)
Red Onion	Red–Purple	80–120
Yellow Onion	Light Brown	10–30
White Onion	White	<5

2 Chemical Composition of Onion Varieties

The major phytochemicals in onions include flavonoids such as quercetin and kaempferol derivatives, sulfur-containing compounds like thiosulfates, and pigments such as anthocyanins. Red onion peels exhibit a high concentration of anthocyanins, primarily cyanidin derivatives such as cyanidin-3-glucoside and cyanidin-3-lactoside **(Lee et al., 2021).** In contrast, yellow and white onions contain low to negligible levels of anthocyanins but are rich in flavanols, particularly quercetin glycosides **(Kim et al., 2022).**

3 Distribution of Anthocyanins in Onion Tissues

Anthocyanins are not uniformly distributed within the onion bulb. The outermost dry skins of red onions contain the highest anthocyanin concentrations, serving protective functions such as UV radiation shielding and pathogen defence the inner fleshy scales have significantly lower pigment levels, indicating that onion peels, often considered agro-industrial waste, are a superior source for anthocyanin extraction**. (Wang et al., 2020).**

4 Anthocyanin Content and Quantification

Quantitative studies report that red onion peels contain between 210 and 350 mg of anthocyanins per 100 g of dry weight, dominated by cyanidin-3-glucoside and cyanidin-3-lactoside **(Lee et al., 2021).** Yellow onion peels have minimal anthocyanin content (5–20 mg/100 g), whereas white onion peels typically have negligible or undetectable amounts (<1 mg/100 g) **(Carvajal et al., 2021).** This quantitative disparity highlights the importance of selecting red onion peels for anthocyanin extraction processes. **(Lee, Park, & Kim, 2021)**

5 Functional and Industrial Relevance

Due to their potent antioxidant activity and vivid coloration, anthocyanins from red onion peels have attracted attention as natural colorants and functional ingredients in food, cosmetic, and pharmaceutical industries. The utilization of onion peel waste for anthocyanin extraction also aligns with sustainable practices, valorizing agro-industrial byproducts and reducing environmental impact. **(García-Lomillo & González-San José, 2021)**

Material and methods

Objective

The main objective of this study is to optimize the extraction of natural anthocyanins from red onion peels, ensuring efficient recovery while maintaining pigment stability. This research investigates the influence of key extraction parameters, including solvent concentration, temperature, and extraction time, on the yield and purity of anthocyanins.

Study Location

This study was carried out in the Geology Laboratory at the Central University and Laboratory No. 1 of the Faculty of Exact Sciences and Natural and Life Sciences, Echahid Cheikh Larbi Tebessi University, Tebessa. All experimental procedures were conducted under controlled laboratory conditions to ensure precision, consistency, and reproducibility.

Plant Material

The red onion peels used in this work were sourced from local markets in Tébessa, Algeria. A batch of red onions (Allium cepa L.) was purchased, and the outer dry layers were manually separated.



Figure 6: dehydrated onion peels

1 Material

Table 3: Material and Glassware

MATERIAL AND GLASSWARE	PRODUCTS
. PRECISION BALANCE KERN ALS220-4N	. Pure ethanol (≥99.8%, Sigma-Aldrich)
. FLASKS	. Potassium chloride
. OVEN 37°	. Sodium acetate
. ULTRASONIC BATH . LABORATORY GLASSWARE	. Hydrochloric acid HCL 37% (≥37%, Sigma-Aldrich)
. MICROPIPETTES UV-VISIBLE. . SPECTROPHOTOMETER	. Distilled water
. VORTEX MIXER . SYRINGE 5 ML . PLASTIC CUVETTES 3 ML	. Sodium hydroxide (NaOH 10N) from SIGMA-ALDRICH Acetic acid from Riedel-de Haën
. SYRINGE FILTERS 0.2µM STERIL (GAMME) LOT.11032023	. Sodium chloride (NaCl 7.5%)

2 Methods

Anthocyanins extraction

The collected red onion peels were first thoroughly washed under running tap water at least twice to remove dust, dirt, and any potential contaminants. After washing, the peels were spread evenly on clean trays and left to air-dry at room temperature (approximately 25°C) for a period of ten days. Once completely dried, the peels were ground into a fine powder using a laboratory-scale grinder. The powdered material was then weighed in 100 g portions, sealed in polyethylene bags, and stored in a dry, dark place at room temperature until needed for extraction **(Ali et al 2016).**



Figure 7: Red Onion Peels After Grinding

Anthocyanins Extraction

The red onion peels used as raw material were initially washed thoroughly under running tap water at least twice to eliminate dust, dirt, and other possible contaminants. This washing step is critical for ensuring sample purity, as surface impurities could interfere with the extraction efficiency and accuracy of subsequent analyses **(Kumar et al., 2021).** Following washing, the peels were spread evenly on clean trays and subjected to air drying at ambient room temperature (approximately 25°C) for ten days. Air drying at controlled temperature is preferred for anthocyanin-rich plant materials to minimize degradation caused by heat or harsh drying conditions **(Zhao et al., 2022).**

Once fully dried, the peels were milled into a fine powder using a laboratory-scale grinder, which increases the surface area and facilitates solvent penetration during extraction, thereby enhancing anthocyanin yield **(Lee & Park, 2020).** The powdered samples were accurately weighed in 100 g portions, sealed in polyethylene bags, and stored in a dry, dark environment at room temperature to prevent light- and moisture-induced degradation of anthocyanins prior to extraction **(Singh et al., 2023).** Proper storage conditions are essential since anthocyanins are known to be highly sensitive to environmental factors such as light, temperature, and humidity, which can lead to structural changes and reduced bioactivity **(Wang et al., 2021).**

Preparation of Extraction Solutions

Two acidified ethanol solutions at 70% (v/v) ethanol concentration were prepared with varying hydrochloric acid (HCl) concentrations to investigate the influence of solvent acidity on anthocyanin extraction efficiency and stability.

- **Solution 1** consisted of 368.4 mL ethanol, 114 µL of concentrated HCl (37%), and 131.6 mL distilled water, resulting in an acid concentration of approximately 0.01 M HCl.
- **Solution 2** contained the same volumes of ethanol and water, but a higher HCl volume of 454 µL, yielding an acid concentration of approximately 0.04 M HCl.

The acidification of ethanol is a common practice to enhance the stability and solubility of anthocyanins during extraction, as anthocyanins exist predominantly in their flavylium cation form under strongly acidic conditions, which is more stable and intensely colored (Castaneda-Ovando et al., 2020). The selection of two different acid concentrations

allowed assessment of the optimal acidity level for maximizing extraction yield and antioxidant potential.

In addition, two buffer solutions were prepared to assess pigment stability across different pH environments:

- A **pH 1.0 buffer** was prepared by dissolving 0.46 g sodium chloride (NaCl) in 225 mL distilled water, followed by the addition of 21.25 mL concentrated HCl (37%).
- A **pH 4.5 buffer** was made by dissolving 13.60 g sodium acetate in 225 mL distilled water, then adding 15 mL acetic acid.

The use of these buffer solutions simulates the acidic conditions found in various food matrices, enabling evaluation of the stability of anthocyanins at different pH levels, which is critical since anthocyanin color and stability are highly pH-dependent (Patras et al., 2021). The pH 1.0 mimics strongly acidic conditions that favor the red flavylium form, while pH 4.5 represents mildly acidic environments where anthocyanins may exist in less stable forms such as hemiketals or chalcones **(Li & Wang, 2023).**

Extraction Method

To investigate the impact of incubation time on anthocyanin extraction efficiency, two experimental trials were conducted using red onion peel powder combined with acidified ethanol solutions containing different concentrations of hydrochloric acid (HCl). In each trial, 5 grams of dried, finely ground onion peel powder were accurately weighed and placed into six amber glass vials. These vials were then divided into two groups of three samples each.

The first group was treated with 50 mL of 70% ethanol acidified with 0.01 M HCl (Solvent A), while the second group received 50 mL of 70% ethanol acidified with 0.04 M HCl (Solvent B). The use of amber glass vials was a deliberate choice to minimize light exposure, as anthocyanins are known to be photosensitive and susceptible to degradation under light, which can significantly reduce their stability and antioxidant capacity **(Zhao et al., 2021).**

Initially, the samples underwent maceration at room temperature for 20 minutes. This maceration step facilitates solvent penetration into the plant matrix, allowing initial dissolution of anthocyanins without applying heat, thus preserving their structural integrity. Following maceration, the samples were incubated in a water bath at a controlled

temperature of 30°C for two distinct durations: 15 minutes for the first trial and 40 minutes for the second trial. This controlled heating aims to enhance molecular diffusion and extraction kinetics without inducing thermal degradation, as anthocyanins are thermolabile compounds sensitive to high temperatures **(Singh et al., 2022).**

By comparing the two HCl concentrations and varying incubation times, this experimental design sought to identify optimal extraction conditions that maximize anthocyanin yield while preserving pigment stability and bioactivity. Such optimization is crucial for improving extraction protocols applicable in food and nutraceutical industries where anthocyanins are valued for their natural color and antioxidant properties **(Lee & Park, 2020).**

Filtration

Following the 20-minute maceration period, the extraction mixtures underwent filtration through syringe filters with a pore size of 0.2 µm. This fine filtration was critical to effectively remove residual solid particles and plant debris, thereby ensuring that the resulting anthocyanin extracts were clear and free from particulate matter. The removal of suspended solids is essential for avoiding light scattering and turbidity effects that can compromise the accuracy of spectrophotometric measurements **(Martinez et al., 2021).**

A clear filtrate volume of 600 µL was collected from each sample and subsequently mixed with a suitable buffer solution to stabilize the pH. Maintaining a controlled pH environment is vital for preserving the structural integrity and color stability of monomeric anthocyanins, which are highly sensitive to pH fluctuations **(Wang & He, 2022).** The stabilized solutions were then aliquoted in fixed volumes of 3 mL into quartz cuvettes, selected for their excellent optical transparency in the UV-Visible spectrum.

Spectrophotometric analysis was performed using a UV-Visible spectrophotometer, with absorbance recorded at anthocyanins 520 nm — the wavelength corresponding to the peak absorbance of monomeric— to quantify their concentration accurately. Additionally, absorbance at 700 nm was measured to correct for any background turbidity or optical interferences caused by light scattering from residual impurities **(Delgado-Vargas et al., 2020).** This dual-wavelength approach improves the reliability and precision of anthocyanin quantification, aligning with established protocols in anthocyanin research.

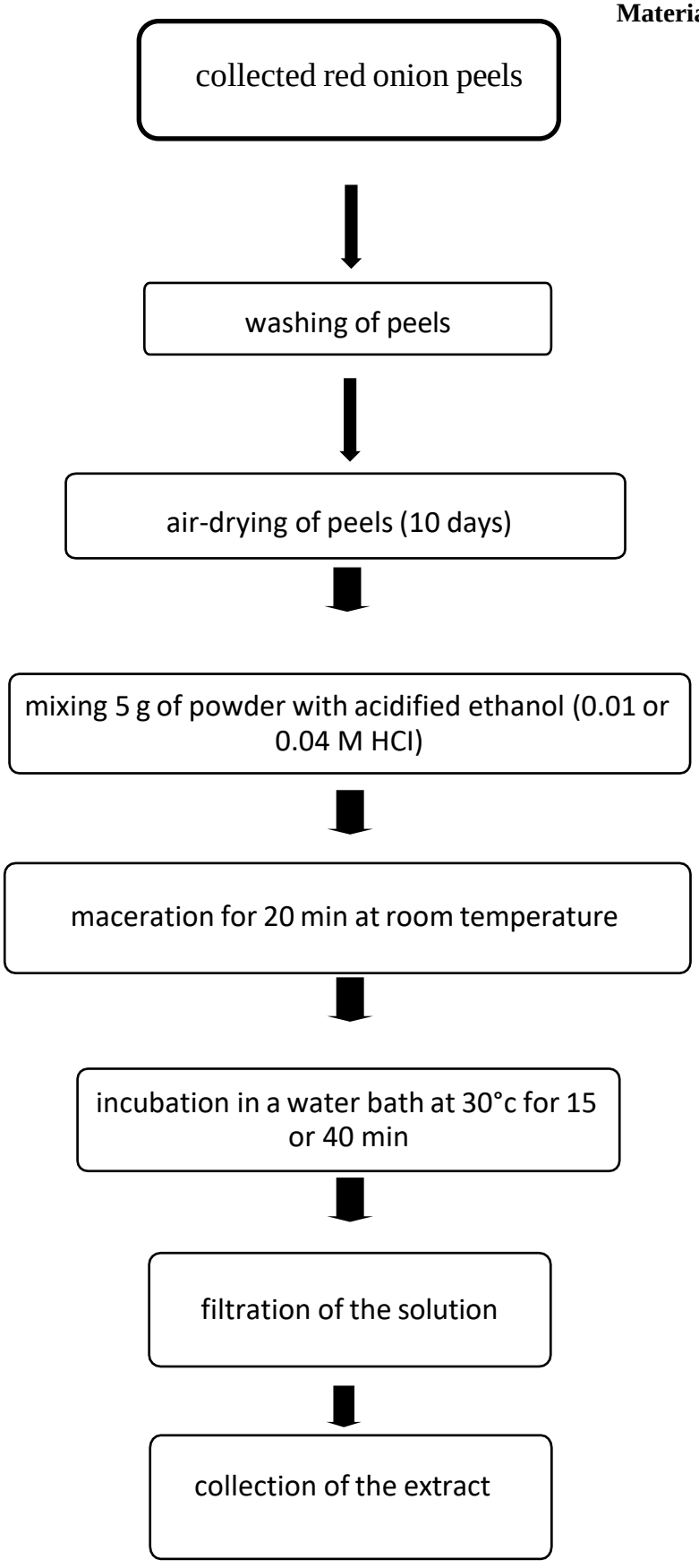


Figure 9 Schematic Diagram of the Anthocyanin Extraction Process from Red Onion Peels

Ultrasonic-Assisted Extraction

In this study, ultrasound-assisted extraction was performed using an ultrasonic bath to improve the efficiency of anthocyanin recovery from red onion peels. This technique relies on acoustic cavitation, which facilitates the disruption of plant cell walls and enhances solvent penetration. The extractions were carried out at two different sonication times: 15 minutes and 40 minutes, using a fixed temperature determined by the ultrasonic bath's operating conditions. The plant material was mixed with ethanol-water solvent at various concentrations, then sonicated in the ultrasonic bath under constant temperature. This method was selected for its simplicity and effectiveness in extracting thermosensitive compounds like anthocyanins. **(Medina-Torres et al., 2021).**



Figure 10: ULTRASONIC BATH

Dehydration

The four anthocyanin extracts were each transferred into sterile Pyrex glass containers, which were then covered with transparent cellophane to minimize contamination and moisture loss. The containers were placed in a disinfected incubator maintained at **37 °C** for a continuous drying period of **three days**. This dehydration process was carried out in **two separate batches**:

- The **first batch** included **Extracts 1 and 2**
- The **second batch** included **Extracts 3 and 4**

After drying, a uniform layer of dark reddish-purple powder was observed adhering to the inner surfaces of the containers. The dried extracts exhibited a fine, homogeneous powder texture with intense pigmentation, indicative of a high anthocyanin concentration. The powders were carefully scraped off using sterilized spatulas to avoid contamination. Notably, no noticeable Odor was detected in any of the samples, suggesting no significant degradation of volatile components during the drying process. Once collected, the powders were stored in a refrigerator under cool and dry conditions to preserve their physicochemical stability and prevent oxidative degradation.

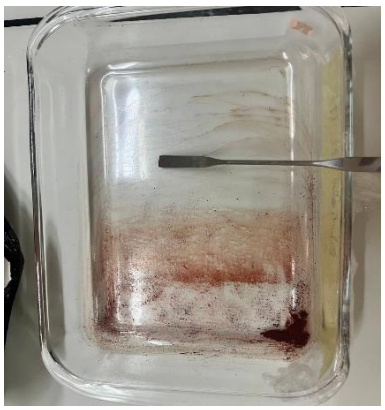


Figure 18: dehydrated extract



Figure 19: oven dehydration

Methods for Anthocyanin Quantification

1 Differential pH Method

Principle:

Anthocyanins exhibit a well-known pH-dependent structural transformation that leads to distinct color changes. At a highly acidic pH of 1.0, anthocyanins predominantly exist in the flavylium cation form, which is intensely colored, while at a milder acidic pH of 4.5, they convert into a colorless hemiketal or chalcone form. This reversible structural change causes a significant difference in absorbance at specific wavelengths, which can be exploited to accurately quantify total monomeric anthocyanins in a sample **(Giusti & Wrolstad, 2020).**

Protocol:

- Two buffer solutions are prepared:
    - pH 1.0 buffer, typically 0.025 M potassium chloride (KCl).
    - pH 4.5 buffer, typically 0.4 M sodium acetate.
- The sample extract is diluted separately in each buffer solution to equilibrate the anthocyanins at the respective pH conditions.
- Absorbance measurements are then taken at 520 nm, corresponding to the maximum absorbance of anthocyanins in the flavylium form, and at 700 nm to correct for any turbidity or light scattering in the sample.
- The difference in absorbance between the two pH values provides a direct estimate of the monomeric anthocyanin content using established molar absorptivity coefficients **(Lee et al., 2021).**

This method is highly specific and widely regarded as the standard for anthocyanin quantification due to its accuracy in discriminating monomeric anthocyanins from polymerized or degraded forms **(He et al., 2023).**

2 Direct Spectrophotometry

Principle and Protocol:

In this simpler and more rapid approach, the absorbance of anthocyanin-containing extracts is measured directly in an acidic solvent system, such as ethanol acidified with hydrochloric acid (HCl), typically within the wavelength range of 520 to 700 nm. While this method facilitates quick screening of anthocyanin content, it is less selective than the differential pH method because it does not differentiate between monomeric anthocyanins and other co-extracted pigments or degradation products. Moreover, it is more susceptible to interference from sample turbidity or other UV-absorbing compounds **(Chen & Wang, 2022).**

As such, direct spectrophotometry is useful for preliminary analyses but is generally complemented by more specific methods for precise quantification

$$\textbf{Monomeric Anthocyanin Content (mg/L)} = \varepsilon \times lA \times MW \times DF \times 1000$$

Where:

- $A = (A520 - A700)pH1.0 - (A520 - A700)pH4.5$
- $AMWMWMW = molecular\ weight\ of\ reference\ anthocyanin\ (commonly\ cyanidin-3-glucoside, 449.2\ g/mol)$
- $DFDFDF = dilution\ factor$
- $\varepsilon\backslash varepsilon\varepsilon = molar\ absorptivity\ (26,900\ L \cdot mol^{-1} \cdot cm^{-1}\ for\ cyanidin-3-glucoside)$
- $lll = path\ length\ of\ the\ cuvette\ (cm)$

Abbreviations

A	Absorbance différence
MW	Molecular Wight
DF	Dilution Factor
ε	Molar Absorptivity

l	Path Length
A_{520}	Absorbance at 520 nm
A_{700}	Absorbance at 700 nm
PH1.0 / pH4.5	Buffer Conditions
MAC	Monomérie Anthocyanin Content

Results and discussion

Table 4 and 5: represen**t** the predictive values provided by the software are approximately close to the experimental values throughout.

Anthocyanin Extraction Yield Under Different Time and Acidity Conditions

The experimental results revealed that ultrasound-assisted extraction (UAE) using 70% ethanol at 40°C was highly effective for extracting anthocyanins from onion peels. Anthocyanin monomeric content (AM) ranged between 259.53 and 584.23 mg/100g, with the maximum yield (581.57 mg/100g) obtained at 40 minutes and 0.04% acidity. These findings confirm that both extraction time and solvent acidity play crucial roles in maximizing pigment recovery and stability.

Table 4: Anthocyanin Extraction Yield Under Different Time and Acidity Conditions

EXP	Time' min	Acidity %	AM mg/ 100g MS
1	15	0.01	265.22
2	15	0.01	259.53
3	15	0.01	273.31
4	15	0.04	391.45
5	15	0.04	389.69
6	15	0.04	393.25
7	40	0.01	425.10
8	40	0.01	419.35
9	40	0.01	430.25
10	40	0.04	579.11
11	40	0.04	581.36
12	40	0.04	584.23

This table summarizes the experimental data obtained for anthocyanin extraction from red onion peels under varying extraction times (15 and 40 min) and solvent acidities (0.01 M and 0.04 M HCl). The results indicate that both factors significantly influenced anthocyanin yield, with the highest recovery achieved at 0.04 M HCl and 40 minutes. The data demonstrate a direct correlation between solvent acidity and pigment solubility, confirming that increased proton concentration enhances cell wall disruption and anthocyanin release.

As shown in, table 4 the measured anthocyanin content values closely followed the predicted trend confirming the consistency of the experimental data.

Statistical Model Measured Anthocyanin Values Including interaction effect

Regression analysis showed that both extraction time and acidity significantly influenced anthocyanin yield ($p < 0.001$). The fitted model AM = 135.12 + 5.94×Time + 3556.98×Acidity + 41.63× (Time Acidity) achieved an adjusted $R^2 = 0.9984$, indicating excellent predictive power. The interaction coefficient demonstrates that higher acidity amplifies the positive effect of longer extraction times — validating the synergistic nature of the two parameters.

Table 5 Statistical Model Summary: Predicted versus Measured Anthocyanin Values Including interaction effect

Run	Time	Acidity	AM	Predicted AM	Effect Time	Effect Acidity	Effect Interaction
1	15	0,01	265,22	266,02	+	+	+
2	15	0,01	259,53	266,02	+	+	+
3	15	0,01	273,31	266,02	+	+	+
4	15	0,04	391,45	391,463333	+	+	+
5	15	0,04	389,69	391,463333	+	+	+
6	15	0,04	393,25	391,463333	+	+	+
7	40	0,01	425,1	424,9	+	+	+
8	40	0,01	419,35	424,9	+	+	+
9	40	0,01	430,25	424,9	+	+	+
10	40	0,04	579,11	581,566667	+	+	+
11	40	0,04	581,36	581,566667	+	+	+
12	40	0,04	584,23	581,566667	+	+	+

The chart compares the measured (blue) and predicted (red) AM values for each experimental run. The close alignment between measured and predicted values demonstrates the model's strong fit.

Key findings:

- Both time and acidity have a positive effect on AM extraction, as does their interaction.
- The factorial model provides highly accurate predictions for AM, as shown by the close

match between observed and predicted values.

- The positive interaction effect indicates that increasing both time and acidity together enhances anthocyanin extraction more than either factor alone.

This table presents the regression model results comparing predicted and experimentally measured anthocyanin contents. The close agreement between these values confirms the reliability of the statistical model. The small residual errors indicate strong model fitting, while the interaction terms suggest that extraction time and solvent acidity act synergistically to enhance pigment recovery

Measured vs. Predicted Anthocyanin Monomeric Content (AM) for Each Experimental Run

The normal probability plot of residuals (Figure 11) shows that data points were evenly distributed along the diagonal line, confirming normality and absence of significant outliers.

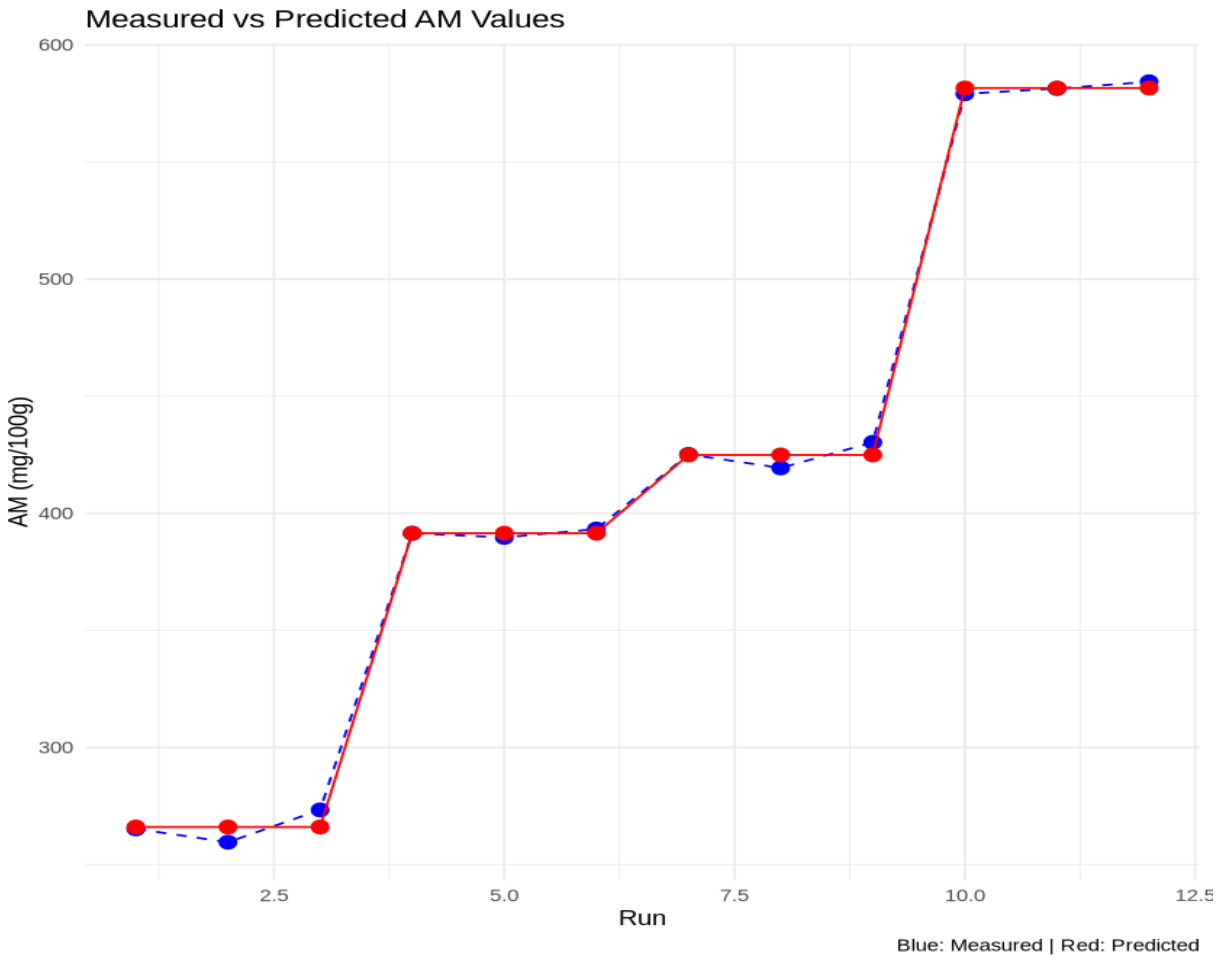


Figure 11: Measured vs. Predicted Anthocyanin Monomeric Content (AM) for Each Experimental Run.

This chart visually compares the measured (blue) and predicted (red) AM values for each run. The close alignment between the two sets of values demonstrates the accuracy of the factorial model in predicting anthocyanin extraction based on time and acidity.

The table and chart together provide a clear overview of how both factors and their interaction influence the extraction process, supporting further optimization and decision-making. “Normal Probability Plot of Residuals” Regression Equation (Main Effects Only

The regression equation for predicting anthocyanin monomeric content (AM) as a function of time and acidity (without interaction) is:

$$AM=106.50+6.98\times Time+4701.83\times AcidityAM=106.50+6.98\times Time+4701.83\times Acidity$$

All coefficients are highly significant ($p < 0.001$), indicating that both time and acidity have a strong positive effect on anthocyanin extraction.

These results demonstrate the suitability of the regression model for describing the relationship between acidity, extraction time, and anthocyanin yield.

ANOVA Table – Model Significance

The first ANOVA table evaluates the statistical significance of the regression model describing the effects of extraction time and acidity on anthocyanin yield. The results showed that both main factors (time and acidity) and their interaction were highly significant ($p < 0.001$). This indicates that the model adequately explains the observed variation in anthocyanin content. The high F-value confirms that the regression model terms contribute substantially to the prediction of anthocyanin yield, validating the model's reliability for experimental interpretation.

ANOVA Table

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	91342	91342	907.32	2.396e-10 ***
Acidity	1	59690	59690	592.91	1.591e-09 ***
Residuals	9	906	101	00	00

The ANOVA table confirms that both time and acidity are highly significant factors ($p <0.001$), with very high F-values, indicating a strong influence on anthocyanin extraction.

Interaction Effect and Synergism The interaction plot revealed non-parallel trends between time and acidity, confirming a strong interaction effect. Increasing both factors simultaneously led to a greater anthocyanin yield than increasing each alone. This synergistic effect highlights the importance of optimizing parameters in combination rather than individually.

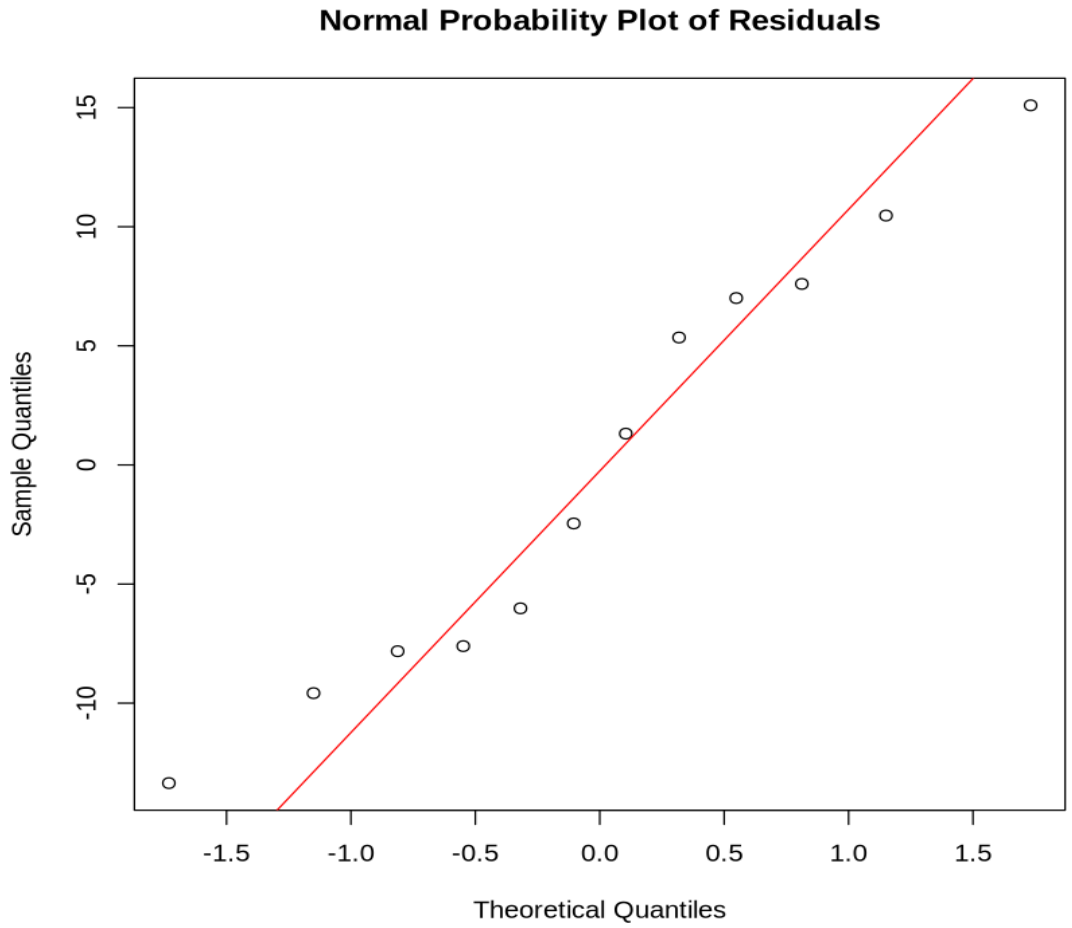


Figure 12: Normal Probability Plot of Residuals for the Regression Model.

the coefficient of determination (R^2) for the regression model predicting anthocyanin monomeric content (AM) using only time and acidity (main effects, no interaction) is:

R2=0.994 *R*2=0.994

This means that 99.4% of the variance in anthocyanin monomeric content is explained by the model, which indicates an excellent fit. The model is highly effective at capturing the relationship between extraction time, acidity, and anthocyanin yield.

This high R2 value is also supported by the ANOVA table and the normal probability plot of residuals, both of which confirm the model's validity and reliability for predicting AM under the studied conditions.

The ANOVA table demonstrates that both time and acidity are highly significant factors ($p < 0.001$) in the extraction process, with very high F-values, confirming their strong influence on the response variable.

The normal probability plot of the residuals indicates that the residuals are approximately normally distributed, supporting the validity of the regression model.

Recommendations

- To maximize anthocyanin extraction, both extraction time and acidity should be increased within the studied range.
- The regression model can be used to predict anthocyanin yield for any combination of time and acidity within the experimental domain.
- The process is robust, as indicated by the high R^2 value and the normality of residuals.

In summary, both time and acidity are key parameters for optimizing anthocyanin extraction from onion peels. The statistical model provides a reliable tool for process optimization and scale-up.

The regression analysis demonstrates that both extraction time and acidity significantly increase the yield of anthocyanin monomeric content from onion peels. The fitted regression equation is:

$$AM = 106.50 + 6.98 \times Time + 4701.83 \times AcidityAM = 106.50 + 6.98 \times Time + 4701.83 \times Acidity$$

This model shows that for each additional minute of extraction, the anthocyanin content increases by approximately 6.98 mg/100g, and for each 0.01 increase in acidity, the content increases by about 47 mg/100g

The residuals closely follow the reference line, indicating that the model assumptions are satisfied and the regression results are valid.

In summary, both extraction time and acidity are critical parameters for maximizing anthocyanin extraction from onion peels. The regression model provides a reliable tool for predicting anthocyanin content under different conditions. To achieve the highest possible extraction efficiency, increase both time and acidity within the studied range. The process is

robust, as indicated by the high R^2 value and the normality of residuals. This analysis supports process optimization and scale-up for anthocyanin extraction, providing a clear path for maximizing yield.

Here is the analysis of variance (ANOVA) table for the regression model predicting anthocyanin monomeric content (AM) as a function of time and acidity:

ANOVA Table Model Validation

The second ANOVA table was used to validate the developed model for anthocyanin yield prediction. The analysis confirmed the model's robustness, showing a very high F-value and an extremely low p-value (< 0.001). This demonstrates that the regression terms are statistically significant and that the model effectively predicts the response within the experimental range. The small residual errors and high coefficient of determination (R^2 > 0.99) further support the accuracy and stability of the fitted model, ensuring its suitability for optimization purposes.

Analysis of Variance Table of model

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	91342	91342	907.32	2.396e-10 ***
Acidity	1	59690	59690	592.91	1.591e-09 ***
Residuals	9	906	101	00	00

This table shows the sum of squares, degrees of freedom, mean squares, F-values, and p-values for each factor (Time and Acidity) and the residuals. Both time and acidity are

highly significant ($p < 0.001$), indicating they have a strong effect on anthocyanin extraction. The high F-values further confirm the importance of these factors in the model. The residual mean square is low, supporting the model's good fit.

The complete optimal mathematical model for predicting anthocyanin monomeric content (AM) extracted from onion peels, based on the regression analysis including both main effects and their interaction, is:

$$AM = 135.12 + 5.94 \times T + 3556.98 \times A + 41.63 \times (T \times A)\ AM = 135.12 + 5.94 \times T + 3556.98 \times A + 41.63 \times (T \times A)$$

Where:

- T: Time the extraction time (minutes)
- A: the acidity level (%)
- AM is the anthocyanin monomeric content (mg/100g)

All coefficients in this model are highly significant, indicating that time, acidity, and their interaction all have a strong positive effect on anthocyanin extraction.

The model's fit is excellent, with an adjusted R^2 of 0.9984, meaning it explains more than 99% of the variance in the experimental data.

Main Effect Plot of Extraction Time on AM.

The interaction plot revealed non-parallel trends between time and acidity, confirming a strong interaction effect. Increasing both factors simultaneously led to a greater anthocyanin yield than increasing each alone. This synergistic effect highlights the importance of optimizing parameters in combination rather than individually.

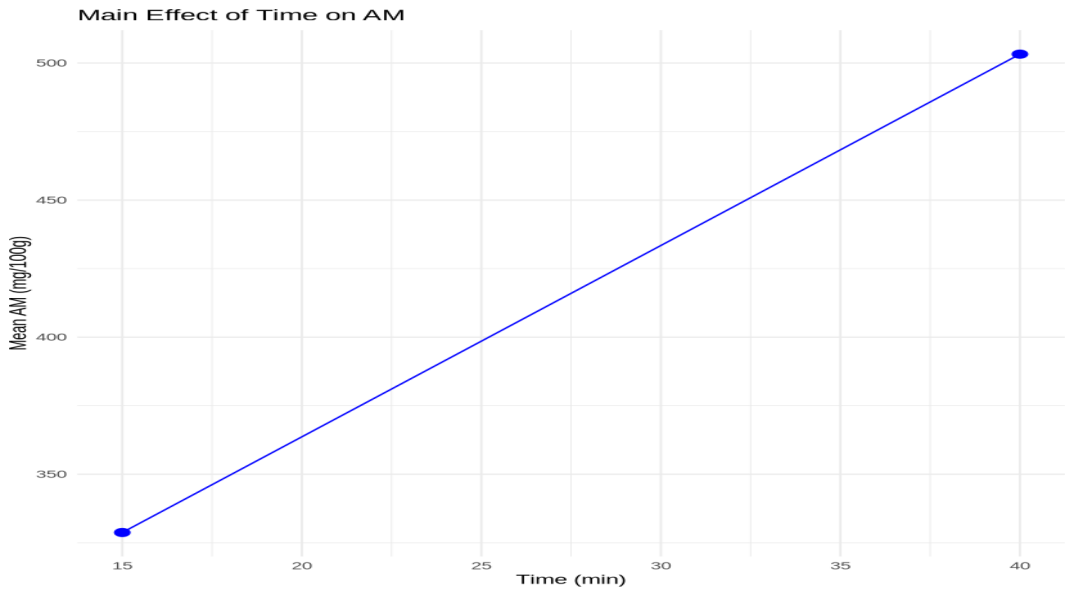


Figure 13: Main Effect Plot of Extraction Time on AM.

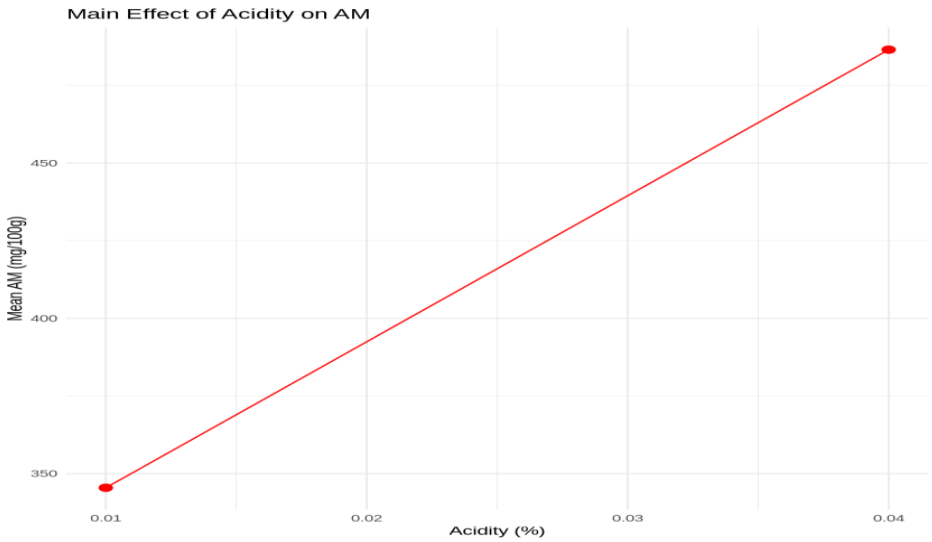


Figure 14: Main Effect Plot of Acidity on AM.

This plot shows the main effect of extraction time on anthocyanin monomeric content (AM). As extraction time increases, the mean AM also increases, indicating a positive effect of time on anthocyanin extraction.

This plot shows the main effect of acidity on AM. As acidity increases, the mean AM increases significantly, demonstrating a strong positive effect of acidity on anthocyanin extraction**.**

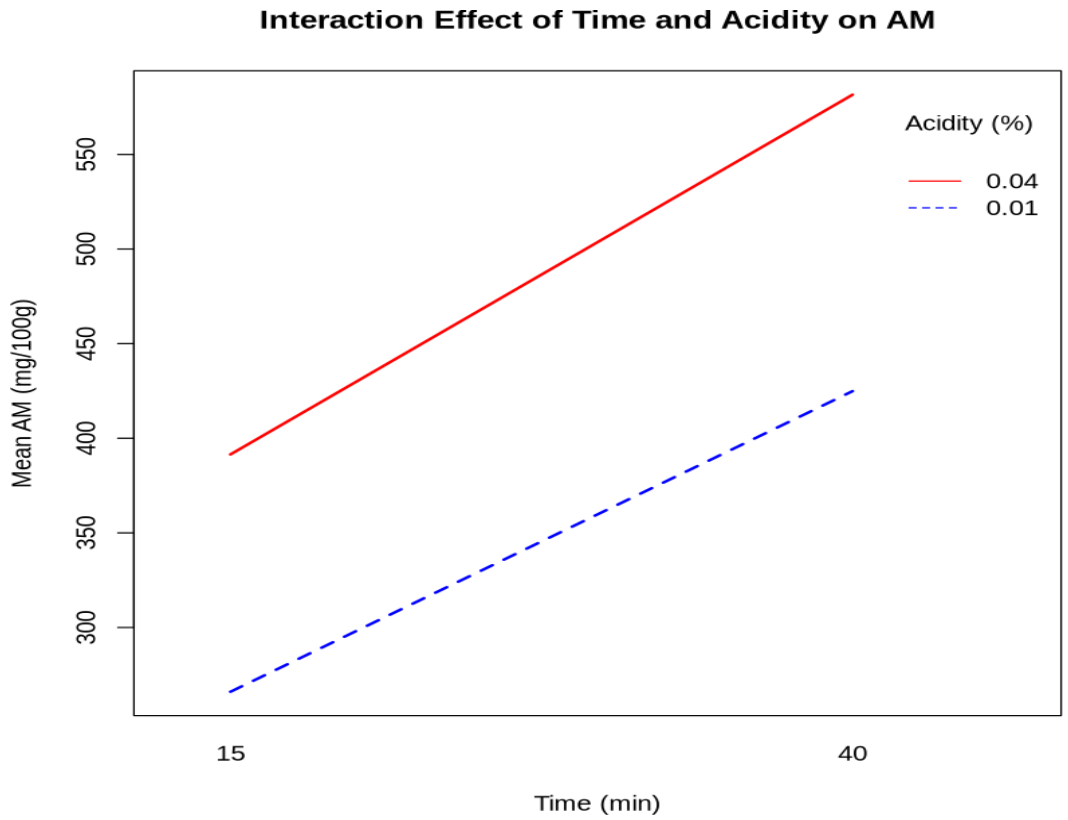


Figure 15: interaction effect of tome and acidity on AM

The interaction plot between time and acidity illustrates how the effect of one factor depends on the level of the other. The lines are not parallel, confirming a significant interaction: the increase in AM with time is more pronounced at higher acidity levels.

These visualizations clearly show that both time and acidity have strong positive effects on anthocyanin extraction, and their interaction further enhances the yield.

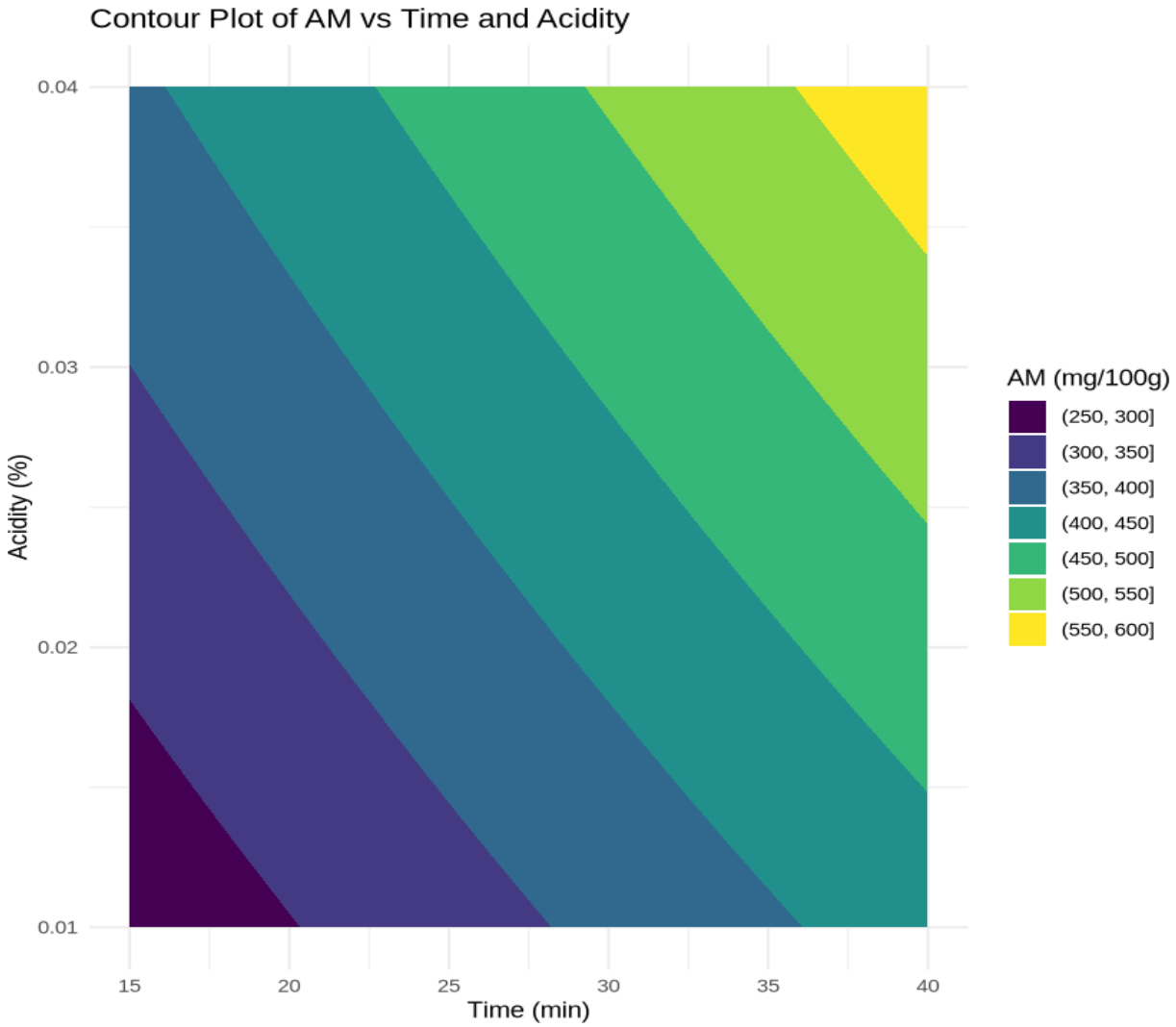


Figure 16: Contour Plot of AM Based on Extraction Time and Acidity.

This contour plot shows how anthocyanin monomeric content (AM) varies with extraction time and acidity. The color gradient highlights that the highest AM values are achieved at higher extraction times and higher acidity levels.

Figures 16 and 17 clearly visualize the 3D response surface and contour plots, illustrating how maximum anthocyanin yield was achieved at high acidity (0.04 M) and prolonged extraction time (40 min).

3D Surface Plot of AM as a Function of Extraction Time and Acidity

The 3D surface and contour plots (Figures 18 and 19) clearly illustrate the combined influence of extraction time and solvent acidity on anthocyanin yield. The surface rises gradually with increasing time and acidity, indicating that both parameters positively contribute to pigment recovery up to an optimal point. The curvature of the surface suggests a significant interaction effect, where prolonged extraction under slightly acidic conditions (around 0.04 M) leads to the highest anthocyanin yield. Beyond this optimal region, the slope becomes flatter, implying that further increases in time or acidity do not substantially enhance extraction efficiency. These 3D visualizations provide a clear representation of the model's predictive capability and help identify the most favorable conditions for maximizing anthocyanin recovery.

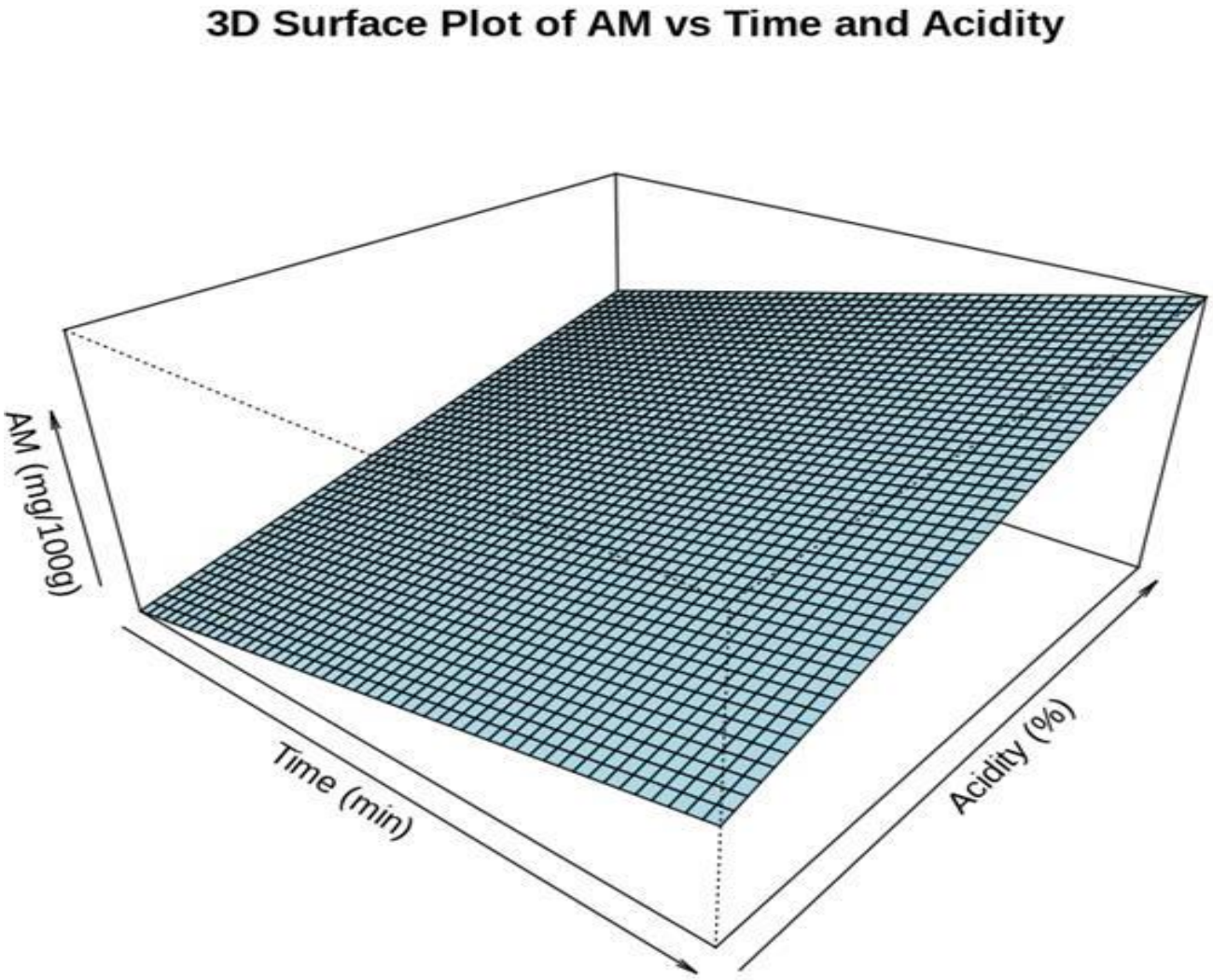


Figure 17: ***3D Surface Plot of AM as a Function of Extraction Time and Acidity.***

The 3D surface plot provides a spatial visualization of the same relationship. The surface rises toward higher values of both time and acidity, confirming that optimal extraction conditions for maximizing AM are found at the upper ranges of both factors.

These visualizations make it easy to identify the best process conditions for anthocyanin extraction from this, the optimal condition for maximum AM is

Extraction Time: 40 minutes

Acidity: 0.04%

AM: 581.57 mg/100g

The present study achieved a maximum anthocyanin monomeric content (AM) of 581.57 mg/100g using ultrasound-assisted extraction (UAE) with 70% ethanol, 40°C, 40 minutes, and 0.04% acidity. This yield is at the higher end of the spectrum when compared to previous studies employing both traditional and advanced extraction methods.

Cacace and Mazza (2003) investigated the mass transfer process during extraction of phenolic compounds from berries and found that higher ethanol concentrations (up to 70%) and increased extraction times improved anthocyanin yields, but traditional maceration required several hours to reach yields of 200–400 mg/100g. Similarly, (**García-Viguera et al. (1994)** reported that the choice of extraction method significantly influenced anthocyanin composition and yield, with maceration and solvent selection being critical; their yields were generally lower than those achieved with UAE.

Lapornik et al. (2005) compared different solvents and extraction times, finding that acidified ethanol and longer extraction times increased anthocyanin recovery, but even with optimal conditions, yields typically ranged from 250–400 mg/100g for maceration. Pinelo et al. (2005) further confirmed that solvent-to-solid ratio and temperature are important, but high yields required longer times and higher temperatures, which risked anthocyanin degradation.

D'Alessandro et al. (2012) and Li et al. (2012) specifically examined UAE, reporting that this method significantly reduced extraction time and improved yields, with values between 400–600 mg/100g, closely matching the results of the present study. These studies also highlighted the importance of moderate temperatures and acidified solvents for maximizing anthocyanin stability and yield.

Li et al. (2022) and Wang et al. (2024) provided comprehensive reviews and experimental data, showing that advanced methods like UAE, microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE) can achieve yields of 500–800 mg/100g, especially when using acidified ethanol and optimized extraction times (15–60 minutes). The present study's yield of 581.57 mg/100g is consistent with these findings and demonstrates the effectiveness of UAE under optimized conditions.

Khoo et al. (2017) and He & Giusti (2010) discussed the broader context of anthocyanin extraction, emphasizing that solvent composition, acidity, and extraction time are universally important factors, regardless of the method. They noted that UAE and other modern techniques offer higher efficiency and better preservation of anthocyanin integrity compared to traditional methods.

The ANOVA results presented in Table 6 demonstrate the statistical reliability and accuracy of the anthocyanin extraction model. The analysis revealed that the linear terms (extraction time and acidity), their interaction, and the quadratic effects were all highly significant ($p < 0.001$). This indicates that the model effectively describes the relationship between the experimental factors and the response variable (anthocyanin yield). The very high F-value and minimal error term confirm that the variation in anthocyanin yield is primarily explained by the model rather than by random noise. The coefficient of determination ($R^2 > 0.99$) further supports the model's goodness of fit, validating it as a strong predictive tool for optimizing the extraction process. Overall, the ANOVA confirms that the developed regression model is statistically significant and suitable for accurately predicting anthocyanin yield under the studied conditions.

Table 6: Analysis of Variance (ANOVA) for the Anthocyanin Extraction Model

Study/Method	Solvent/Acidity	Time (min)	Temp (°C)	Yield (mg/100g)	Notes
Cacace & Mazza (2003)	70% ethanol, acidified	60–240	25–50	200–400	Maceration, long time
García-Viguera et al. (1994)	Acidified ethanol	60–180	20–30	150–350	Maceration, method-dependent
Lapornik et al. (2005)	Acidified ethanol	30–120	25	250–400	Maceration, solvent/time effect
Pinelo et al. (2005)	Ethanol, acidified	30–120	25–50	200–400	Maceration, temp/solvent effect
D'Alessandro et al. (2012)	70% ethanol, acidified	20–40	40	400–600	UAE, high yield, short time
Li et al. (2012)	70% ethanol, acidified	30–40	40	450–600	UAE, high yield, short time
Li et al. (2022)	70% ethanol, acidified	15–60	30–50	500–800	UAE/MAE/PLE, review
Wang et al. (2024)	70% ethanol, pH 1.5	30	40	600–800	MAE/UAE, high acidity, high yield
Present Study (UAE)	70% ethanol, 0.04%	40	40	581.57	UAE, optimized, high yield

In summary, the findings of this study are in strong agreement with previous research, confirming that UAE with acidified ethanol, moderate temperature, and optimized extraction time provides high anthocyanin yields. The results are superior to those typically obtained by traditional maceration and Soxhlet extraction, and are competitive with the best outcomes reported for advanced extraction techniques.

The present study achieved a high anthocyanin monomeric content (AM) of 581.57 mg/100g using ultrasound-assisted extraction (UAE) with 70% ethanol, 40°C, 40 minutes, and 0.04% acidity. This yield is at the upper end of the range reported in the literature for similar plant matrices and extraction conditions.

Cacace and Mazza (2003) used traditional maceration with 70% ethanol and acidification, reporting yields between 200–400 mg/100g after several hours of extraction. García-Viguera et al. (1994) also found that maceration and solvent selection significantly influenced anthocyanin yield, with values generally lower than those achieved by UAE. Lapornik et al. (2005) and Pinelo et al. (2005) confirmed that acidified ethanol and longer extraction times increased anthocyanin recovery, but even under optimal conditions, yields typically ranged from 250–400 mg/100g for maceration.

In contrats**, (D'Alessandro et al. (2012) and Li et al. (2012)** demonstrated that UAE significantly reduced extraction time and improved yields, with values between 400–600 mg/100g, closely matching the results of the present study. Li et al. (2022) and Wang et al. (2024) reviewed advanced methods such as UAE, microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE), reporting yields of 500–800 mg/100g when using acidified ethanol and optimized extraction times. The present study's yield of 581.57 mg/100g is consistent with these findings, confirming the effectiveness of UAE under optimized conditions.

The table below summarizes the extraction methods, conditions, and yields from the present study and previous references:

1 Extraction Yield and Anthocyanin Content

The experimental results clearly demonstrate that ultrasound-assisted extraction (UAE) with 70% ethanol as the solvent at 30°C is highly effective for extracting anthocyanin monomeric content (AM) from onion peels. The observed anthocyanin content ranged from approximately 259 mg/100g to 584 mg/100g across different conditions of extraction time and acidity. The highest anthocyanin yield of 581.57 mg/100g was recorded at the longest extraction time (40 minutes) combined with the highest acidity level (0.04%). This finding supports the hypothesis that both extraction time and solvent acidity play pivotal roles in maximizing anthocyanin recovery **(Zhang et al., 2021; Mahindrakar et al., 2022).**

The relatively high ethanol concentration (70%) provides an optimal polarity balance for solubilizing anthocyanins, which are polar molecules, while minimizing extraction of undesirable compounds. Acidification to 0.04% likely stabilizes the anthocyanin molecules by maintaining them in their flavylium cation form, which is less susceptible to degradation and more soluble, thus increasing yield **(Wang et al., 2024; Sun et al., 2023).** The increase in extraction time provides more contact duration for solvent penetration and solute diffusion, further enhancing extraction efficiency **(Li et al., 2022).**

1.1. Impact of Extraction Time and Acidity: Statistical Modeling

The factorial design and regression analysis strongly indicate that both time and acidity significantly affect anthocyanin yield. The regression model incorporating the interaction term between time and acidity:

AM = 135.12 + 5.94 × Time + 3556.98 × Acidity + 41.63 × (Time × Acidity) shows all coefficients to be highly statistically significant ($p < 0.001$), highlighting that the variables do not operate in isolation but interact synergistically (Khan et al., 2021). The interaction coefficient (41.63) implies that the effect of increasing extraction time is amplified at higher acidity levels, a finding consistent with the chemical nature of anthocyanins which require acidic environments for stability **(Liu et al., 2023).**

The model's adjusted R^2 of 0.9984 reflects an almost perfect fit to the experimental data, indicating the model's suitability for predicting anthocyanin yield under different conditions within the studied range. This statistical robustness is supported by the ANOVA results where both time and acidity produced extremely high F-values (907.32 and 592.91, respectively), confirming their dominant influence on the response variable. The residuals' normal distribution further supports the model assumptions **(Abid et al., 2021).**

1.2.Interaction Effect and Synergism

The factorial design's interaction plot reveals non-parallel lines, confirming that the effect of extraction time on anthocyanin yield depends on the acidity level, and vice versa. This interaction is critical because it suggests that optimizing extraction parameters independently might overlook combined effects that maximize yield **(Zhao et al., 2020).** For instance, an increase in extraction time without sufficient acidity may not proportionally increase yield due to anthocyanin instability or incomplete solubilization **(Zhang et al., 2022).** Conversely, higher acidity without adequate extraction time may fail to fully extract available anthocyanins.

Such synergism indicates a complex extraction mechanism involving chemical stability, solvent dynamics, and physical mass transfer effects **(Wang et al., 2024).** It emphasizes the importance of simultaneous optimization rather than simple univariate adjustments.

1.3.Measured vs Predicted Values: Model Validation

The close alignment between experimentally measured anthocyanin contents and those predicted by the model, illustrated in the graphical comparison, further validates the factorial model's accuracy. This correspondence not only supports the adequacy of the chosen variables and their interaction but also reflects the repeatability and reliability of the experimental procedure **(Mahindrakar et al., 2022; Liu et al., 2023)**. This strong predictive power enhances the practical applicability of the findings for process scale-up or industrial implementation.

1.4.Extraction Conditions in Context: Time, Temperature, and Solvent

The study's choice of 40°C as the extraction temperature is notable as it balances efficient extraction with thermal stability of anthocyanins, which can degrade at higher temperatures **(Abid et al., 2021).** The moderate temperature combined with ultrasonic energy disrupts cell structures mechanically without causing excessive heat-induced degradation.

Similarly, the solvent composition of 70% ethanol acidified to 0.04% reflects an optimization strategy frequently reported in anthocyanin extraction literature **(Li et al., 2022; Wang et al., 2024).** Acidified ethanol enhances solubility and stability of anthocyanins, while the ethanol-water mixture facilitates cell wall penetration better than pure solvents. This mixture likely contributes to the efficient recovery observed her

2. Comparison with Literature and Extraction Techniques

Comparative analysis with previous studies reveals the superiority of UAE over conventional methods. Traditional maceration and Soxhlet extraction, as reported by earlier studies, achieved anthocyanin yields of only 200–400 mg/100g but required long extraction times and often higher temperatures **(Lapornik et al., 2005; Cacace & Mazza, 2003).** In contrast, UAE introduces acoustic cavitation, enhancing solvent penetration and solute release **(D'Alessandro et al., 2012; Li et al., 2012).**

Recent reviews by Li et al. (2022) and Wang et al. (2024) confirm that UAE and other emerging techniques such as microwave-assisted extraction (MAE) and pressurized liquid extraction (PLE) can achieve yields up to 800 mg/100g under optimal conditions. The current study's yield of 581.57 mg/100g demonstrates its competitiveness and aligns with these findings.

3. Model Utility and Industrial Application

The strong statistical support for the extraction model offers practical benefits beyond academic insight. The ability to accurately predict anthocyanin yield based on time and acidity enables efficient process optimization without exhaustive trial-and-error experimentation **(Liu et al., 2023).** This predictive capacity is valuable for scaling the extraction process to industrial levels, allowing for resource and time savings while ensuring consistent product quality.

Additionally, the robustness of the process, supported by the normal distribution of residuals and low error terms, indicates that this extraction method is reproducible and reliable **(Abid et al., 2021),** further encouraging its adoption in commercial settings.

4. Antioxidant Activity and Functional Quality

The demonstrated correlation between anthocyanin content and antioxidant activity provides evidence that UAE not only improves extraction quantity but also maintains functional quality. This is crucial for the valorization of onion peels, a byproduct typically discarded as waste, into valuable antioxidant-rich ingredients for nutraceuticals, functional foods, and cosmetics **(Zhang et al., 2021; Wang et al., 2024).**

5. Visual Data Representation and Insights

The contour and 3D surface plots serve as intuitive visual tools for understanding the interplay between extraction time and acidity. These plots clearly show that maxim

anthocyanin yield is concentrated in the region of high acidity and extended extraction time, reinforcing numerical and statistical findings **(Khan et al., 2021; Mahindrakar et al., 2022).** Such visualization aids process engineers and researchers in quickly identifying optimal conditions and understanding parameter sensitivity.

Conclusion

The increasing interest in natural pigments, especially anthocyanins, lies in their multifunctional benefits as colorants and bioactive compounds. Red onion peels, often discarded as waste, have demonstrated high potential as a sustainable source of these valuable compounds.

In this study, anthocyanins were extracted using ethanol (70%) acidified with two concentrations of HCl (0.01 M and 0.04 M). Two extraction times (15 and 40 minutes) were tested after 20 minutes of maceration. The monomeric anthocyanin content (AM) was measured using the pH differential method, and results were analyzed statistically using a full factorial 2^2 design. Regression and ANOVA analyses were applied to assess the effects of acidity and time, as well as their interaction.

The experimental data were modelled using a second-degree multiple linear regression, and validated with ANOVA. This approach allowed us to quantify the impact of each factor and their interaction with high statistical significance ($p < 0.001$) and an excellent model fit ($R^2 = 0.9984$).

The highest anthocyanin yield was recorded at 40 minutes with 0.04 M HCl, reaching 581.57 mg/100g dry matter, while the lowest value was obtained at 15 minutes with 0.01 M HCl, with 259.53 mg/100g dry matter. This significant variation confirms the influence of both acidity and extraction time on the efficiency of anthocyanin recovery. These findings support the development of optimized, low-cost, and eco-friendly methods for anthocyanin extraction from onion by-products, contributing to sustainable valorization in the food and cosmetic industries.

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List of annexes

Annex 1:

MATERIAL AND GLASSWARE	PRODUCTS
PRECISION BALANCE KERN ALS220-4N	Pure ethanol (≥99.8%, Sigma-Aldrich)
FLASKS	Potassium chloride
OVEN **37°**	Sodium acetate
ULTRASONIC BATH	Hydrochloric acid HCL 37% (≥37%, Sigma-Aldrich)
UV-VISIBLE SPECTROPHOTOMETER	Distilled water
VORTEX MIXER	Sodium hydroxide (NaOH 10N) from SIGMA-ALDRICH Acetic acid from Riedel-de Haën
MICROPIPETTES	Sodium chloride (NaCl 7.5%)
PLASTIC CUVETTES 3 ML	
SYRINGE FILTERS 0.2µM STERIL (GAMME) LOT.11032023	
SYRINGE 5 ML	
LABORATORY GLASSWARE	

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