เม็ดสีพืช: มากกว่าสารให้สี สู่ทรัพยากรชีวภาพมูลค่าสูงจากของเหลือในอุตสาหกรรมอาหาร

บทคัดย่อ

เม็ดสีธรรมชาติจากพืชกำลังได้รับความสนใจเพิ่มขึ้นในอุตสาหกรรมอาหาร เครื่องสำอาง และเภสัชภัณฑ์ เนื่องจากผู้บริโภคต้องการผลิตภัณฑ์ที่มาจากธรรมชาติ ปลอดภัย และเป็นมิตรต่อสิ่งแวดล้อมมากขึ้น เม็ดสีพืชไม่ได้ทำหน้าที่เพียงสร้างสีสันเท่านั้น แต่ยังเกี่ยวข้องกับการสังเคราะห์ด้วยแสง การป้องกันความเสียหายจากแสง การต้านอนุมูลอิสระ การตอบสนองต่อความเครียด และการดึงดูดสัตว์ผสมเกสร

เม็ดสีพืชที่มีศักยภาพเชิงอุตสาหกรรมประกอบด้วยแอนโทไซยานิน คลอโรฟิลล์ แคโรทีนอยด์ และบีตาเลน แหล่งวัตถุดิบที่น่าสนใจคือเปลือก กาก และเศษเหลือจากการแปรรูปผักและผลไม้ ซึ่งมีต้นทุนต่ำและสามารถนำมาเพิ่มมูลค่าตามแนวคิดเศรษฐกิจหมุนเวียน อย่างไรก็ตาม เม็ดสีธรรมชาติมักเสื่อมสภาพจากความร้อน แสง ออกซิเจน และความเป็นกรด–ด่าง จึงจำเป็นต้องพัฒนาเทคโนโลยีการสกัดที่เป็นมิตรต่อสิ่งแวดล้อมควบคู่กับวิธีเพิ่มความคงตัว เช่น การห่อหุ้มระดับไมโครและนาโน การสร้างนาโนอิมัลชัน และการเกิดโคพิกเมนเทชัน บทความนี้อธิบายบทบาท แหล่งที่มา วิธีสกัด การเพิ่มความคงตัว และความท้าทายของการนำเม็ดสีพืชไปใช้ในระดับอุตสาหกรรม โดยเรียบเรียงจากบทความปริทัศน์ของ Klavins และคณะ พ.ศ. 2569

คำสำคัญ: เม็ดสีธรรมชาติ แอนโทไซยานิน คลอโรฟิลล์ แคโรทีนอยด์ บีตาเลน การสกัดสีเขียว เศรษฐกิจหมุนเวียน

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1. สีของพืชไม่ได้มีไว้เพื่อความสวยงามเท่านั้น

เมื่อมองดูดอกไม้สีแดง ผลไม้สีม่วง ใบไม้สีเขียว หรือผลสุกสีส้ม เรามักเข้าใจว่าสีเหล่านี้มีหน้าที่ดึงดูดสายตา แต่ในทางชีววิทยา เม็ดสีเป็นองค์ประกอบสำคัญที่ช่วยให้พืชดำรงชีวิตอยู่ได้

เม็ดสีบางชนิดทำหน้าที่รับพลังงานแสงเพื่อใช้ในการสังเคราะห์ด้วยแสง บางชนิดช่วยระบายพลังงานส่วนเกิน ป้องกันเยื่อหุ้มเซลล์และคลอโรพลาสต์จากอนุมูลอิสระ ขณะที่สีของดอกและผลช่วยดึงดูดแมลงผสมเกสรและสัตว์ที่ช่วยกระจายเมล็ด นอกจากนี้ พืชมักสร้างเม็ดสีเพิ่มขึ้นเมื่อเผชิญความแห้งแล้ง อุณหภูมิสูงหรือต่ำ รังสีอัลตราไวโอเลต การขาดธาตุอาหาร หรือการเข้าทำลายของเชื้อโรค

แผนภาพในหน้า 3 ของบทความต้นฉบับแสดงให้เห็นว่าเม็ดสีทั้งสี่กลุ่มมีหน้าที่เชื่อมโยงกันหลายด้าน ตั้งแต่การสังเคราะห์ด้วยแสง การต้านอนุมูลอิสระ การป้องกันรังสีอัลตราไวโอเลต ไปจนถึงการตอบสนองต่อความแห้งแล้งและอุณหภูมิ

2. เม็ดสีพืชสี่กลุ่มสำคัญ

กลุ่มเม็ดสี	สีที่พบโดยทั่วไป	ตัวอย่างแหล่งอาหาร	บทบาทเด่นในพืช	จุดอ่อนด้านความคงตัว
แอนโทไซยานิน	แดง ม่วง น้ำเงิน	เบอร์รี องุ่น กะหล่ำปลีม่วง มันม่วง	ต้านอนุมูลอิสระ ป้องกันรังสี UV และดึงดูดสัตว์ผสมเกสร	สีเปลี่ยนมากตามค่า pH
คลอโรฟิลล์	เขียว	ผักโขม คะน้า บรอกโคลี หญ้าธัญพืช	รับพลังงานแสงเพื่อการสังเคราะห์ด้วยแสง	เสื่อมจากกรด ความร้อน แสง และออกซิเจน
แคโรทีนอยด์	เหลือง ส้ม แดง	แครอต ฟักทอง มะเขือเทศ พริก ข้าวโพด	ช่วยรับแสง ป้องกันความเสียหายจากแสง และต้านอนุมูลอิสระ	ออกซิไดซ์และเปลี่ยนไอโซเมอร์ได้ง่าย
บีตาเลน	เหลืองส้ม แดงม่วง	บีตรูต แก้วมังกร กระบองเพชร ผักโขมแดง	ต้านอนุมูลอิสระ ปรับสมดุลออสโมซิส และตอบสนองต่อความเครียด	ไวต่อความร้อน แสง ออกซิเจน และโลหะบางชนิด

ภาพในหน้า 6–7 ของบทความต้นฉบับเปรียบเทียบโครงสร้าง แหล่งอาหาร และช่วงสีของเม็ดสีแต่ละกลุ่ม ทำให้เห็นชัดว่าโครงสร้างทางเคมีเป็นตัวกำหนดทั้งสี การละลาย และความคงตัวของเม็ดสี

2.1 แอนโทไซยานิน: สีที่เปลี่ยนตามความเป็นกรด–ด่าง

แอนโทไซยานินเป็นสารกลุ่มฟลาโวนอยด์ที่ละลายน้ำ พบมากในแวคิวโอลของเซลล์พืช ทำให้ดอก ผล ใบ และหัวใต้ดินมีสีแดง ม่วง หรือน้ำเงิน ตัวอย่างที่พบได้ง่าย ได้แก่ องุ่นดำ บลูเบอร์รี ราสป์เบอร์รี กะหล่ำปลีม่วง และมันเทศสีม่วง

หน้าที่สำคัญของแอนโทไซยานินคือช่วยดูดกลืนรังสีอัลตราไวโอเลต ทำหน้าที่คล้ายแผ่นกรองแสง ลดการเกิดความเสียหายต่อคลอโรพลาสต์ และกำจัดอนุมูลอิสระ พืชจึงมักสะสมแอนโทไซยานินเพิ่มขึ้นเมื่อได้รับแสงจัด ขาดน้ำ ขาดไนโตรเจนหรือฟอสฟอรัส หรือถูกเชื้อโรคเข้าทำลาย

สมบัติที่โดดเด่นที่สุดคือสีเปลี่ยนตามค่า pH ในสภาพกรดจัด โมเลกุลอยู่ในรูปฟลาวิเลียมแคตไอออนและให้สีแดง เมื่อ pH เพิ่มขึ้นอาจเปลี่ยนเป็นม่วง น้ำเงิน หรือไม่มีสี หากอยู่ในสภาพด่างมาก โครงสร้างอาจเปิดวงและสูญเสียสีอย่างถาวร สมบัตินี้เป็นทั้งข้อจำกัดและโอกาส เพราะสามารถนำไปพัฒนาเป็นฉลากบรรจุภัณฑ์อัจฉริยะที่เปลี่ยนสีตามความสดของอาหารได้

2.2 คลอโรฟิลล์: หัวใจของการสังเคราะห์ด้วยแสง

คลอโรฟิลล์เป็นเม็ดสีที่ละลายในไขมัน พบในเยื่อไทลาคอยด์ของคลอโรพลาสต์ โครงสร้างประกอบด้วยวงพอร์ไฟรินที่มีแมกนีเซียมอยู่ตรงกลาง และมีสายไฟทอลที่ช่วยยึดโมเลกุลไว้กับเยื่อหุ้ม

คลอโรฟิลล์ดูดกลืนแสงสีน้ำเงินและสีแดง แต่สะท้อนแสงสีเขียว จึงทำให้ใบไม้ปรากฏเป็นสีเขียว คลอโรฟิลล์เอและคลอโรฟิลล์บีดูดกลืนแสงต่างช่วงกันเล็กน้อย ช่วยให้พืชใช้พลังงานแสงได้กว้างขึ้น นอกจากรับพลังงานแสงแล้ว ระบบรงควัตถุยังช่วยระบายพลังงานส่วนเกินเป็นความร้อน เพื่อลดความเสียหายจากแสงมากเกินไป

ข้อจำกัดสำคัญคือคลอโรฟิลล์ไม่คงตัวในสภาพกรด เมื่อแมกนีเซียมหลุดออกจากวงพอร์ไฟรินจะเกิดฟีโอไฟติน ซึ่งมีสีเขียวหม่นหรือน้ำตาลอมเขียว ปรากฏการณ์นี้อธิบายได้ว่าทำไมผักสีเขียวที่ต้มเป็นเวลานานหรือสัมผัสกรดจึงสูญเสียสีเขียวสด

2.3 แคโรทีนอยด์: เม็ดสีที่ช่วยป้องกันแสง

แคโรทีนอยด์เป็นเม็ดสีที่ละลายในไขมัน แบ่งเป็นสองกลุ่มใหญ่ ได้แก่

แคโรทีน ซึ่งประกอบด้วยคาร์บอนและไฮโดรเจน เช่น บีตาแคโรทีนและไลโคปีน และ **แซนโทฟิลล์** ซึ่งมีออกซิเจนอยู่ในโมเลกุล เช่น ลูทีน ซีแซนทิน และแอสตาแซนทิน

แคโรทีนอยด์ดูดกลืนแสงในช่วงประมาณ 400–500 นาโนเมตร แล้วถ่ายทอดพลังงานให้คลอโรฟิลล์ จึงช่วยขยายช่วงแสงที่พืชใช้ในการสังเคราะห์ด้วยแสง ขณะเดียวกันยังช่วยดับออกซิเจนสถานะซิงเกิลและอนุมูลอิสระที่เกิดขึ้นในระบบสังเคราะห์ด้วยแสง แซนโทฟิลล์บางชนิดมีบทบาทในวัฏจักรแซนโทฟิลล์ ซึ่งเปลี่ยนพลังงานแสงส่วนเกินให้เป็นความร้อน

แคโรทีนอยด์บางชนิด เช่น บีตาแคโรทีน เป็นสารตั้งต้นของวิตามินเอในมนุษย์ อย่างไรก็ตาม พันธะคู่ต่อเนื่องจำนวนมากในโมเลกุลทำให้ไวต่อออกซิเจน แสง และความร้อน การออกซิเดชันทำให้สีซีดลง ขณะที่ความร้อนอาจทำให้โครงสร้างแบบทรานส์เปลี่ยนเป็นแบบซิส ส่งผลต่อสีและการนำไปใช้ประโยชน์ของร่างกาย

2.4 บีตาเลน: สีแดงและสีเหลืองที่พบเฉพาะในพืชบางกลุ่ม

บีตาเลนเป็นเม็ดสีที่มีไนโตรเจน พบเด่นในพืชบางวงศ์ของอันดับ Caryophyllales แบ่งเป็นสองประเภท คือ **บีตาไซยานิน** ซึ่งให้สีแดงถึงม่วง และ **บีตาแซนทิน** ซึ่งให้สีเหลืองถึงส้ม ตัวอย่างแหล่งสำคัญ ได้แก่ บีตรูต แก้วมังกรเนื้อแดง กระบองเพชร และผักโขมแดง

ข้อสังเกตทางชีววิทยาที่น่าสนใจคือ ยังไม่พบพืชชนิดใดที่สังเคราะห์แอนโทไซยานินและบีตาเลนตามธรรมชาติพร้อมกัน แม้เม็ดสีทั้งสองจะมีสีและหน้าที่คล้ายกันก็ตาม แอนโทไซยานินสังเคราะห์จากฟีนิลอะลานีน ส่วนบีตาเลนมีไทโรซีนเป็นสารตั้งต้น

บีตาเลนมีความคงตัวในช่วง pH ประมาณ 3–7 จึงเหมาะกับอาหารที่เป็นกรดอ่อนหรือใกล้เป็นกลางมากกว่าแอนโทไซยานิน แต่ยังเสื่อมได้จากความร้อน แสง ออกซิเจน และไอออนโลหะ

3. เปลี่ยนของเหลือจากอาหารให้เป็นแหล่งเม็ดสีมูลค่าสูง

อุตสาหกรรมแปรรูปผักและผลไม้สร้างเปลือก เมล็ด กาก และเศษพืชจำนวนมาก วัสดุเหล่านี้มักถูกนำไปทำปุ๋ย อาหารสัตว์ หรือกำจัดเป็นของเสีย ทั้งที่ยังมีเม็ดสีและสารออกฤทธิ์ทางชีวภาพเหลืออยู่ในปริมาณสูง

ตัวอย่างวัตถุดิบที่มีศักยภาพ ได้แก่

- กากราสป์เบอร์รี บลูเบอร์รี แบล็กเคอร์แรนต์ และองุ่น เป็นแหล่งแอนโทไซยานิน
- เปลือกหอมแดง ซังข้าวโพดม่วง และเปลือกผลไม้สีม่วง เป็นแหล่งแอนโทไซยานินและสารฟีนอลิก
- เศษผักโขม บรอกโคลี และใบผัก เป็นแหล่งคลอโรฟิลล์
- เปลือกแครอต ฟักทอง ส้ม มะเขือเทศ และพริก เป็นแหล่งแคโรทีนอยด์
- เปลือกและกากบีตรูต รวมถึงเปลือกแก้วมังกร เป็นแหล่งบีตาเลน

การใช้วัสดุเหล่านี้ช่วยลดปริมาณของเสีย ลดต้นทุนวัตถุดิบ และสร้างผลิตภัณฑ์มูลค่าสูง เช่น สีผสมอาหารธรรมชาติ สารต้านอนุมูลอิสระ ส่วนผสมอาหารเชิงหน้าที่ เครื่องสำอาง และวัสดุบรรจุภัณฑ์ นับเป็นตัวอย่างที่ชัดเจนของเศรษฐกิจชีวภาพหมุนเวียน

4. การสกัดสีเขียว: ได้เม็ดสีมากขึ้น ใช้ทรัพยากรน้อยลง

4.1 การสกัดด้วยตัวทำละลายแบบดั้งเดิม

เป็นวิธีที่ทำได้ง่ายและมีต้นทุนอุปกรณ์ไม่สูง แอนโทไซยานินและบีตาเลนเหมาะกับน้ำ เอทานอล หรือตัวทำละลายมีขั้ว ส่วนคลอโรฟิลล์และแคโรทีนอยด์ต้องใช้ตัวทำละลายที่มีความเป็นขั้วน้อยกว่า

เอทานอลได้รับความสนใจมากเพราะเหมาะกับผลิตภัณฑ์อาหารมากกว่าตัวทำละลายบางชนิด อย่างไรก็ตาม การสกัดแบบดั้งเดิมอาจใช้เวลานาน ใช้ตัวทำละลายมาก และหากใช้อุณหภูมิสูงเกินไปอาจทำให้เม็ดสีเสื่อม

4.2 การสกัดด้วยอัลตราซาวนด์

คลื่นอัลตราซาวนด์ทำให้เกิดฟองขนาดเล็กในของเหลว เมื่อฟองยุบตัวจะเกิดแรงเฉือนที่ช่วยทำลายผนังเซลล์ ตัวทำละลายจึงเข้าสู่เนื้อเยื่อและพาเม็ดสีออกมาได้เร็วขึ้น

ข้อดีคือช่วยลดเวลา ปริมาณตัวทำละลาย และพลังงาน แต่หากใช้กำลังสูงหรือเป็นเวลานานเกินไป อนุมูลอิสระและความร้อนเฉพาะจุดอาจทำลายเม็ดสี โดยเฉพาะแอนโทไซยานิน

4.3 การสกัดด้วยไมโครเวฟ

ไมโครเวฟทำให้โมเลกุลน้ำและไอออนในเนื้อเยื่อพืชเคลื่อนที่อย่างรวดเร็ว เกิดความร้อนภายในเซลล์และทำให้ผนังเซลล์แตก วิธีนี้สกัดได้รวดเร็วและใช้พลังงานอย่างมีประสิทธิภาพ เหมาะกับคลอโรฟิลล์ แคโรทีนอยด์ และบีตาเลนจากเปลือกหรือเศษพืช

ข้อควรระวังคือการควบคุมอุณหภูมิ หากเกิดความร้อนสูงเกินไป เม็ดสีอาจออกซิไดซ์หรือสลายตัว

4.4 การใช้เอนไซม์

เอนไซม์ เช่น เซลลูเลส เพกทิเนส และเฮมิเซลลูเลส ช่วยย่อยองค์ประกอบของผนังเซลล์ ทำให้เม็ดสีถูกปลดปล่อยออกมาได้ง่ายขึ้น วิธีนี้ใช้สภาวะไม่รุนแรงและอาจใช้ร่วมกับอัลตราซาวนด์ได้ แต่มีต้นทุนเอนไซม์และต้องควบคุม pH กับอุณหภูมิให้เหมาะสม

4.5 สนามไฟฟ้าแบบพัลส์และพลาสมาเย็น

สนามไฟฟ้าแบบพัลส์ทำให้เยื่อหุ้มเซลล์เกิดรูพรุนชั่วคราว เม็ดสีจึงแพร่ออกจากเซลล์ได้ง่าย ส่วนพลาสมาเย็นสร้างอนุภาคพลังงานสูงที่ช่วยทำลายโครงสร้างผนังเซลล์โดยไม่ต้องให้ความร้อนสูง

เทคนิคเหล่านี้เหมาะกับเม็ดสีที่ไวต่อความร้อน แต่ยังต้องพัฒนาอุปกรณ์และประเมินผลกระทบของสารออกซิแดนต์ที่อาจเกิดขึ้นระหว่างกระบวนการ

4.6 การสกัดด้วยคาร์บอนไดออกไซด์เหนือวิกฤต

เมื่อคาร์บอนไดออกไซด์อยู่ภายใต้อุณหภูมิและความดันเหนือจุดวิกฤต จะมีสมบัติคล้ายทั้งของเหลวและก๊าซ จึงแทรกซึมเข้าไปในวัตถุดิบและละลายสารที่ไม่ชอบน้ำได้ดี เหมาะอย่างยิ่งสำหรับแคโรทีนอยด์

ข้อดีคือได้สารสกัดที่มีตัวทำละลายตกค้างน้อย สามารถปรับความจำเพาะด้วยความดันและอุณหภูมิ แต่เครื่องมือมีราคาสูง จึงเหมาะกับผลิตภัณฑ์ที่มีมูลค่ามาก

4.7 ตัวทำละลายยูเทคติกเชิงลึกจากธรรมชาติ

ตัวทำละลายชนิดนี้ผลิตโดยผสมสารธรรมชาติสองชนิดขึ้นไป เช่น กรดอินทรีย์ น้ำตาล หรือโคลีน ให้เกิดของเหลวที่มีจุดหลอมเหลวต่ำ สามารถปรับคุณสมบัติให้เหมาะกับเม็ดสีแต่ละชนิดได้

ข้อดีคือมีศักยภาพในการย่อยสลายทางชีวภาพ มีความเป็นพิษต่ำ และลดการใช้ตัวทำละลายปิโตรเลียม ข้อจำกัดคือมีความหนืดสูง ซึ่งอาจลดการถ่ายโอนมวลเมื่อขยายกระบวนการสู่ระดับอุตสาหกรรม เทคโนโลยีอัลตราซาวนด์ ไมโครเวฟ ของไหลเหนือวิกฤต และตัวทำละลายยูเทคติกจึงมีศักยภาพสูง แต่ต้องปรับสภาวะให้เหมาะกับวัตถุดิบและเม็ดสีแต่ละชนิด

5. เหตุใดสีธรรมชาติจึงซีดง่าย

ความไม่คงตัวเป็นอุปสรรคสำคัญที่สุดของการใช้เม็ดสีธรรมชาติในผลิตภัณฑ์จริง ปัจจัยที่ต้องควบคุมประกอบด้วย

แสง ซึ่งกระตุ้นโมเลกุลให้เกิดปฏิกิริยาออกซิเดชัน
ออกซิเจน ซึ่งทำลายระบบพันธะคู่และทำให้สีซีด
ความร้อน ซึ่งเร่งปฏิกิริยาการสลายตัวและเปลี่ยนโครงสร้าง
ค่า pH ซึ่งเปลี่ยนประจุและโครงสร้างของเม็ดสี
ไอออนโลหะ ซึ่งอาจเร่งออกซิเดชันหรือสร้างสารเชิงซ้อน

องค์ประกอบของอาหาร เช่น ไขมัน โปรตีน น้ำตาล และเกลือ ซึ่งอาจช่วยป้องกันหรือเร่งการเสื่อม
กิจกรรมของน้ำ ซึ่งมีผลต่อการเคลื่อนที่ของสารและอัตราการเกิดปฏิกิริยา

เม็ดสีแต่ละกลุ่มตอบสนองต่อปัจจัยเหล่านี้แตกต่างกัน จึงไม่มีวิธีเพิ่มความคงตัวแบบเดียวที่ใช้ได้กับเม็ดสีทุกชนิด

6. เทคโนโลยีเพิ่มความคงตัวของเม็ดสี

6.1 การห่อหุ้มระดับไมโคร

การห่อหุ้มคือการนำเม็ดสีไว้ภายในวัสดุป้องกัน เช่น มอลโทเดกซ์ทริน กัมอารบิก เพกทิน แอลจิเนต เวย์โปรตีน หรือโปรตีนจากพืช จากนั้นทำให้แห้งด้วยการพ่นฝอยหรือการทำแห้งเยือกแข็ง

ชั้นห่อหุ้มช่วยลดการสัมผัสกับออกซิเจน แสง ความชื้น และความร้อน ทำให้เก็บรักษาได้นานขึ้น การห่อหุ้มคลอโรฟิลล์ด้วยโปรตีนและคาร์โบไฮเดรตสามารถให้ประสิทธิภาพการห่อหุ้มสูงประมาณร้อยละ 90–97 โดยการพ่นฝอยมีข้อได้เปรียบด้านการขยายกำลังการผลิต ส่วนการทำแห้งเยือกแข็งมักให้การป้องกันแสงและ pH ที่ดี แต่มีต้นทุนสูงกว่า

6.2 นาโนอิมัลชันและระบบนำส่งระดับนาโน

เม็ดสีที่ละลายในไขมัน เช่น คลอโรฟิลล์และแคโรทีนอยด์ สามารถกระจายในหยดน้ำมันขนาดนาโนที่แขวนลอยในน้ำ ระบบนี้ช่วยเพิ่มการกระจายตัว ลดการรวมกลุ่มของโมเลกุล และอาจเพิ่มการดูดซึมในระบบทางเดินอาหาร

อย่างไรก็ตาม ประสิทธิภาพขึ้นอยู่กับชนิดของน้ำมัน สารลดแรงตึงผิว ขนาดหยด และองค์ประกอบของผลิตภัณฑ์ การประเมินความปลอดภัยและการเปลี่ยนแปลงระหว่างการย่อยจึงมีความสำคัญ

6.3 โคพิกเมนเทชัน

โคพิกเมนเทชันคือการที่เม็ดสีเกิดปฏิสัมพันธ์กับโมเลกุลอื่น เช่น สารฟีนอลิก โปรตีน พอลิแซ็กคาไรด์ หรือไอออนโลหะ ทำให้โครงสร้างที่ให้สีถูกป้องกันจากน้ำและสารออกซิแดนต์

วิธีนี้เหมาะกับแอนโทไซยานินและบีตาเลน การใช้โคพิกเมนเทชันร่วมกับการห่อหุ้มสามารถช่วยเพิ่มทั้งความเข้มสีและการคงสีระหว่างการเก็บรักษาได้ดีกว่าการใช้วิธีใดวิธีหนึ่งเพียงอย่างเดียว

6.4 การดัดแปลงโครงสร้างทางเคมี

การเติมหมู่อะซิลหรือหมู่เมทิลให้แอนโทไซยานินสามารถเพิ่มความคงตัวต่อแสง ความร้อน และ pH และอาจทำให้ละลายในระบบไขมันได้ดีขึ้น แต่กระบวนการดังกล่าวมีต้นทุนสูงและผลิตภัณฑ์ที่ดัดแปลงแล้วต้องผ่านการประเมินความปลอดภัย

สำหรับคลอโรฟิลล์ สามารถแทนที่แมกนีเซียมตรงกลางโมเลกุลด้วยสังกะสีหรือทองแดง เพื่อสร้างอนุพันธ์ที่ทนกรดและความร้อนได้ดีขึ้น อย่างไรก็ตาม ต้องพิจารณาข้อกำหนดทางกฎหมายของแต่ละประเทศและปริมาณโลหะที่ใช้

6.5 การเติมสารต้านอนุมูลอิสระและสารจับโลหะ

การเติมกรดแอสคอร์บิก โทโคฟีรอล หรือสารฟีนอลิกบางชนิดลงในระบบห่อหุ้มอาจช่วยลดการออกซิเดชันของแคโรทีนอยด์ ขณะที่กรดซิตริกหรือสารจับโลหะช่วยลดผลของไอออนโลหะต่อบีตาเลน

อย่างไรก็ตาม สารเติมแต่งอาจส่งผลต่อรสชาติ กลิ่น และสีของผลิตภัณฑ์ จึงต้องประเมินร่วมกันทั้งด้านความคงตัวและการยอมรับของผู้บริโภค การผสมสารต้านอนุมูลอิสระกับวัสดุห่อหุ้มช่วยเพิ่มความคงตัวของแคโรทีนอยด์ได้ แต่ปฏิสัมพันธ์ระหว่างเม็ดสี วัสดุห่อหุ้ม และสารต้านอนุมูลอิสระยังต้องศึกษาเพิ่มเติม

6.6 การออกแบบบรรจุภัณฑ์และการเก็บรักษา

แม้จะเพิ่มความคงตัวของเม็ดสีแล้ว การบรรจุและเก็บรักษายังคงมีความสำคัญ ผลิตภัณฑ์ควรใช้ภาชนะที่ป้องกันแสงและออกซิเจน อาจใช้บรรยากาศไนโตรเจนหรือบรรจุภัณฑ์สุญญากาศสำหรับแคโรทีนอยด์ รวมทั้งควบคุมอุณหภูมิและความชื้นให้เหมาะสม

7. การประยุกต์ใช้ในผลิตภัณฑ์

อาหารและเครื่องดื่ม

เม็ดสีธรรมชาติสามารถใช้ในโยเกิร์ต เครื่องดื่ม ขนมอบ ลูกกวาด ไอศกรีม ซอส และอาหารพิมพ์สามมิติ นอกจากให้สีแล้ว ยังอาจเพิ่มสมบัติต้านอนุมูลอิสระ แต่ต้องทดสอบว่ารสชาติ เนื้อสัมผัส และสีคงอยู่ตลอดอายุผลิตภัณฑ์หรือไม่

อาหารเชิงหน้าที่และผลิตภัณฑ์เสริมอาหาร

แอนโทไซยานิน แคโรทีนอยด์ และบีตาเลนมีฤทธิ์ต้านอนุมูลอิสระที่รายงานในงานวิจัย จึงมีศักยภาพเป็นส่วนประกอบของอาหารเชิงหน้าที่ อย่างไรก็ตาม การมีฤทธิ์ในหลอดทดลองไม่ได้หมายความว่าจะให้ผลเหมือนกันในร่างกาย จำเป็นต้องศึกษาการย่อย การดูดซึม เมแทบอลิซึม และปริมาณที่เหมาะสม

เครื่องสำอาง

เม็ดสีสามารถใช้ให้สีในผลิตภัณฑ์แต่งหน้า ครีม และผลิตภัณฑ์ดูแลผิว การปรับความสามารถในการละลายด้วยระบบห่อหุ้มหรือนาโนอิมัลชันช่วยให้เม็ดสีที่ไม่ละลายน้ำสามารถกระจายตัวในสูตรเครื่องสำอางได้ดีขึ้น

บรรจุภัณฑ์อัจฉริยะ

แอนโทไซยานินเปลี่ยนสีตาม pH จึงสามารถนำมาผสมในฟิล์มชีวภาพเพื่อเป็นตัวบ่งชี้ความสด เมื่ออาหารเสื่อมสภาพและสร้างสารที่ทำให้ pH เปลี่ยน สีของฟิล์มจะเปลี่ยนตาม ทำให้ผู้บริโภคประเมินคุณภาพอาหารได้ด้วยตาเปล่า

8. ความท้าทายจากห้องปฏิบัติการสู่โรงงาน

แม้งานวิจัยจำนวนมากแสดงว่าเทคโนโลยีสีเขียวช่วยเพิ่มผลผลิตและลดการใช้ตัวทำละลาย แต่ผลสำเร็จในระดับห้องปฏิบัติการยังไม่รับประกันว่าจะใช้ได้ในโรงงานขนาดใหญ่ ประเด็นที่ต้องพิจารณามีดังนี้

ความแตกต่างของวัตถุดิบ

กากหรือเปลือกผลไม้แต่ละฤดูกาลมีปริมาณน้ำ เม็ดสี น้ำตาล และสารฟีนอลิกไม่เท่ากัน จึงต้องมีระบบตรวจสอบและปรับสภาวะการสกัดให้สม่ำเสมอ

ต้นทุนและการใช้พลังงาน

เทคนิคที่ให้ผลสกัดสูงอาจต้องใช้อุปกรณ์ราคาแพง เช่น ระบบความดันสูง สนามไฟฟ้า หรือพลาสมาเย็น จึงต้องเปรียบเทียบรายได้จากผลิตภัณฑ์กับต้นทุนการลงทุน พลังงาน การทำให้บริสุทธิ์ และการกำจัดตัวทำละลาย

ความคงตัวในอาหารจริง

เม็ดสีที่คงตัวในสารละลายทดลองอาจเสื่อมเมื่อผสมในนม โยเกิร์ต เครื่องดื่ม หรือเครื่องสำอาง เพราะผลิตภัณฑ์จริงประกอบด้วยโปรตีน ไขมัน เกลือ เอนไซม์ และจุลินทรีย์หลายชนิด จึงต้องทดสอบในผลิตภัณฑ์จริงตลอดอายุการเก็บรักษา

ความปลอดภัยและกฎระเบียบ

สารสกัดจากของเหลือทางการเกษตรอาจมีสารกำจัดศัตรูพืช โลหะหนัก สารก่อภูมิแพ้ หรือจุลินทรีย์ปนเปื้อน กระบวนการผลิตจึงต้องมีมาตรฐานด้านแหล่งวัตถุดิบ การทำให้บริสุทธิ์ และการควบคุมคุณภาพ นอกจากนี้ คำว่า “ธรรมชาติ” ไม่ได้หมายความว่าปลอดภัยโดยอัตโนมัติ โดยเฉพาะเมื่อใช้สารสกัดเข้มข้นหรือดัดแปลงโครงสร้าง

การยอมรับของผู้บริโภค

ผู้บริโภคอาจยอมรับสีธรรมชาติได้ดี แต่ยังคาดหวังว่าสีต้องสวย สม่ำเสมอ ไม่ทำให้รสชาติเปลี่ยน และมีราคาไม่สูงเกินไป การพัฒนาผลิตภัณฑ์จึงต้องพิจารณาคุณภาพทางประสาทสัมผัสควบคู่กับประโยชน์ด้านสุขภาพและสิ่งแวดล้อม

9. ทิศทางการวิจัยในอนาคต

การพัฒนาเม็ดสีธรรมชาติควรมองเป็นระบบตั้งแต่วัตถุดิบจนถึงผลิตภัณฑ์ ไม่ควรพิจารณาเฉพาะผลผลิตของการสกัด การวิจัยในอนาคตควรให้ความสำคัญกับการทดลองระดับนำร่อง การประเมินต้นทุนเชิงเทคนิคและเศรษฐศาสตร์ การประเมินวัฏจักรชีวิต และการกำหนดมาตรฐานความปลอดภัยร่วมกัน

อีกประเด็นหนึ่งคือการทำความเข้าใจปฏิสัมพันธ์ระหว่างเม็ดสีกับวัสดุห่อหุ้มในระดับโมเลกุล เช่น แรงไฮโดรโฟบิก พันธะไฮโดรเจน การแพร่ของออกซิเจน และเส้นทางการสลายตัว ความรู้นี้จะช่วยให้สามารถออกแบบระบบป้องกันที่เหมาะกับเม็ดสีและผลิตภัณฑ์แต่ละประเภทได้อย่างแม่นยำ

เทคโนโลยีการสกัดแบบอัลตราซาวนด์ ไมโครเวฟ ของไหลเหนือวิกฤต และตัวทำละลายยูเทคติกสามารถลดการใช้ตัวทำละลายที่เป็นพิษและลดภาระจากของเสีย แต่ยังมีข้อจำกัดด้านการขยายกำลังการผลิต เงินลงทุน และเครื่องมือเฉพาะทาง การนำไปใช้จริงจึงต้องหาสมดุลระหว่างผลผลิต คุณภาพ ความคงตัว ต้นทุน และผลกระทบต่อสิ่งแวดล้อม

บทสรุป

เม็ดสีพืชเป็นสารชีวภาพที่มีหน้าที่หลากหลายกว่าการให้สี แอนโทไซยานินช่วยป้องกันรังสีและเปลี่ยนสีตาม pH คลอโรฟิลล์เป็นศูนย์กลางของการรับพลังงานแสง แคโรทีนอยด์ช่วยขยายช่วงการดูดกลืนแสงและป้องกันความเสียหายจากแสง ส่วนบีตาเลนช่วยต้านอนุมูลอิสระและตอบสนองต่อความเครียดของพืช

เปลือก กาก และเศษเหลือจากอุตสาหกรรมอาหารเป็นแหล่งเม็ดสีราคาต่ำที่สามารถนำมาเพิ่มมูลค่าได้ เทคโนโลยีการสกัดสีเขียวช่วยลดเวลา พลังงาน และการใช้ตัวทำละลาย ขณะที่การห่อหุ้ม นาโนอิมัลชัน โคพิกเมนเทชัน และการออกแบบบรรจุภัณฑ์ช่วยเพิ่มความคงตัว

อย่างไรก็ตาม ความสำเร็จเชิงพาณิชย์ไม่ได้ขึ้นอยู่กับการสกัดเม็ดสีให้ได้มากที่สุดเพียงอย่างเดียว แต่ต้องทำให้เม็ดสีมีความปลอดภัย คงตัว ใช้งานได้จริง มีต้นทุนเหมาะสม และเป็นที่ยอมรับของผู้บริโภค การบูรณาการวิทยาศาสตร์พืช เคมีอาหาร วิศวกรรมกระบวนการ นาโนเทคโนโลยี และเศรษฐศาสตร์จึงเป็นกุญแจสำคัญที่จะเปลี่ยนของเหลือทางการเกษตรให้กลายเป็นผลิตภัณฑ์สีธรรมชาติที่มีมูลค่าสูงและยั่งยืน

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The multifunctionality of plant pigments: roles, recovery from by-products and stability challenges

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ABSTRACT

The growing demand for natural pigments in food, cosmetic, and pharmaceutical applications is driven by increasing concerns over the safety, sustainability, and environmental impact of synthetic dyes. Natural pigments derived from plants, algae, and microbial sources offer functional and health-promoting properties but are often limited by intrinsic instability and challenges related to scalable and sustainable production. This review provides a comprehensive overview of major plant pigment groups - anthocyanins, carotenoids, chlorophylls, and betalains, with particular emphasis on their biological roles, chromatic properties, and stability characteristics focusing on the valorisation of food processing by-products and agro-industrial wastes as abundant and low-cost pigment sources within a circular bioeconomy framework. Recent advances in green and non-thermal extraction technologies, including ultrasound-, microwave-, enzyme-assisted extraction, pulsed electric fields, supercritical fluid extraction, and natural deep eutectic solvents, are critically discussed in terms of efficiency, sustainability, and applicability. In addition, current stabilisation strategies, such as microencapsulation, nanoemulsions, and copigmentation, are reviewed as complementary approaches to enhance pigment functionality and application potential. Regulatory inconsistencies and consumer acceptance further shape innovation uptake. Bridging laboratory advances with industrial practice requires pilot-scale validation, techno-economic assessment, and harmonised safety standards.

1. Introduction

Humans love to live in a colourful environment as it resembles nature from which we all are coming: bright colours of flowers, greens of leaves, and the blue of the sky. Since prehistoric times, natural plant origin substances have been used to make food, clothing, our environment colourful, to resemble natural colours; however, colour of natural substances is fast fading and not stable. A breakthrough happened in the 19th century with the invention of synthetic pigments, with a burst of diverse applications across many fields. However, from a contemporary perspective, the majority of synthetic pigments produced in the 19th to mid-20th centuries can nowadays be considered dangerous, many of which as highly toxic poisons, with only a few still in use [1]. So, the search for new, stable and safe pigment sources is continuing, with the focus of attention being on natural plant pigments abundant in nature. Natural pigments are associated with a healthy lifestyle and sustainable consumption; their use can reduce reliance on synthetic materials or even fully replace them [2], supporting development of nutraceuticals, cosmeceuticals, and other products not only with aesthetic, but also functional properties. To develop practically applicable solutions of natural biomass pigments, further developments must meet 2 preconditions:

(1) Stability of natural pigments – natural pigments are produced to serve distinctive functions, their stability is low, as bright colours are prone to rapid fading. To develop natural pigments for practical applications, they should be stabilised, and their stability should be increased (colour fading decreased). Natural pigment stabilisation includes their stability at increased temperatures, in the pH range needed for real-life applications. Natural pigments usually are stable at a specific, narrow pH range; however, for applications, pH stability increase is an essential precondition, just as stability with respect to oxidants;
(2) Abundant and cheap sources of plant biomass – prospective for production to address the need for natural pigments with diverse applications in the food industry, cosmetics, beverages, etc.

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Considering the need to produce bulk amounts of natural pigments, the cost of the source biomass and availability must be considered, as well as the solvents used to align with circularity and sustainability principles, with priority given to industry side stream utilisation.

Application possibilities and stabilisation of natural pigments have been a topic of several review articles [3–7], primarily concentrating on stabilisation approaches, use of whole plant biomass, and encapsulation. A considerable resource for natural pigments is the food processing side streams – peels of fruits and vegetables, press residues obtained as a waste after juice processing, and other plant and microbial biomass processing wastes – largely underutilised for natural pigment production. Consideration of critical factors with respect to natural pigment applications can support breakthroughs in their use and the replacement of synthetic pigments.

The aim of the present study was to review most prospective groups of natural pigments from practical application potential, establishing links between pigment inherent stability, functions in plants, and extraction possibilities of food industry side streams, as well as stabilisation possibilities.

2. Methodology

The review focused on natural plant pigments (anthocyanins, betalains, carotenoids, and chlorophylls) recovered from various types of food industry side streams and agro-waste. A comprehensive literature search was conducted using SCOPUS, SemanticScholar, GoogleScholar, PubMed, and WoS databases (Fig. 1). The article screening process and eligibility assessment, including the criteria used for article selection, are shown in Fig. 1. Highly relevant and influential papers were chosen outside of the predetermined range of publication years.

Relevance of review topics within the field was assessed using data gathered from PubMed and visualised using VOSviewer. Articles with keywords “natural” AND “pigments” published in the period from 2019 to 2025 were gathered, author keywords of the gathered articles (8162 articles) were grouped by their occurrence, visualisation was prepared from the 50 most common keywords appearing at least 10 times within the used set of data. Fig. 2 indicates the relevance of the investigated pigment groups and the related stabilisation and application efforts based on the biological activity (Fig. 2).

3. The role and properties of natural pigments in plants

3.1. Natural pigment function in plants

3.1.1. Anthocyanins

Anthocyanins are water-soluble plant secondary metabolites belonging to the flavonoid family. They are widely recognised and known as the most abundant natural plant pigments, providing different shades of red, purple, and blue in flowers, fruits, and vegetables. Research on anthocyanins can be considered to have begun in the 17th century when the anthocyanins' ability to change colour in acidic medium was first described in Robert Boyle's "Experiments upon Colours" [8]. The name anthocyanins was first coined in 1835 by a German pharmacist, Ludwig Clamor Marquart, who is considered to be the discoverer of this molecule [9]. Anthocyanins are synthesised in plants via the phenylpropanoid pathway followed by the flavonoid biosynthesis pathway with additional structural modifications like acylation, methylation, and glycosylation that improve the stability of anthocyanins. To date, 27 anthocyanidin aglycones are known, of which only 6 are represented in more than 90% of the reported occurrences of anthocyanins [10]. The most commonly found anthocyanidin classes are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin

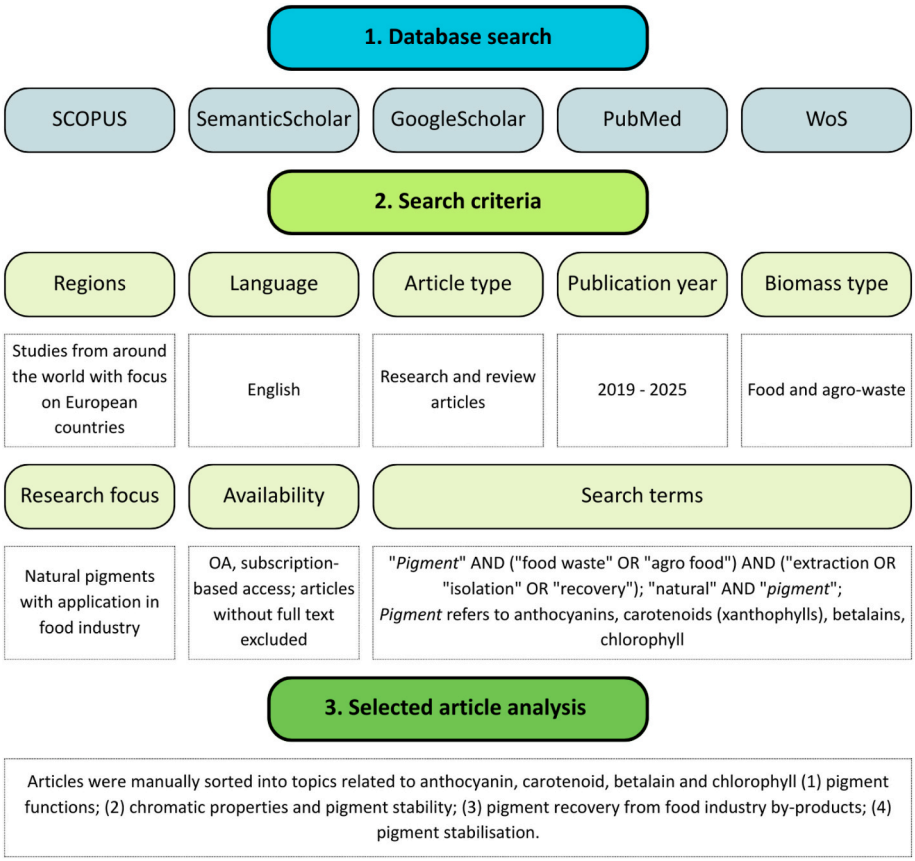

Fig. 1. Methodological flowchart for data extraction from scientific databases used in this review.

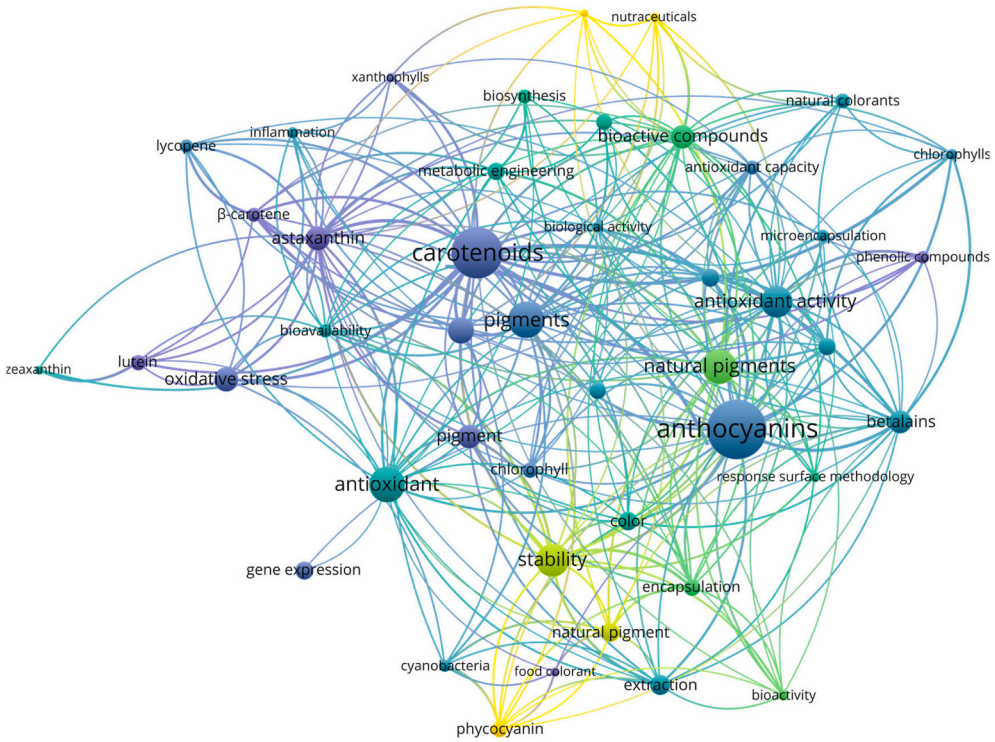

Fig. 2. Network of keywords from 2019 to 2025 regarding search terms "natural" AND "pigments", indicating the importance of topics related to natural pigment research. Visual material prepared using VOSviewer software.

derivatives. The derivatives, which are decorated with carbohydrate, aliphatic or aromatic moieties, are called anthocyanins – these are the naturally occurring pigments distributed throughout the plant kingdom with more than 635 known structures [11,12].

Anthocyanins are stored within the vacuoles of the cells and they can be widely distributed within the plants' organs. Anthocyanins can be present in the epidermal cells, roots, stems, tubers, rhizomes, buds, leaves, vascular tissue, hypocotyls and the expression can also vary greatly. Similar to their distribution, their functions are very multifaceted. Anthocyanin synthesis in various cell types is upregulated as a response to environmental stress like increased light, UV-B radiation, drought, temperature extremes, deficiency of main nutrients (nitrogen and phosphorus), biotic stress from infections, wounding, and pollution [13,14]. Upregulated anthocyanin synthesis is considered to be a way for the plant to mitigate the negative effects caused by abiotic or biotic stressors. Due to the exposure to UV radiation, plants tend to synthesize UV-absorbing phenolic compounds to mitigate the negative effects of the exposure [13]. Since anthocyanins are accumulated in vacuoles, they can act as optical filters, protecting the mesophilic cells and avoiding the photodamage of chloroplasts. Anthocyanins have been reported to be effective free radical scavengers, and therefore elevated biosynthesis contributes to the mitigation of UV exposure [13]. Anthocyanins also possess the ability to chelate different metals, which are then stored in the vacuoles or cytosol to protect the plant under metal

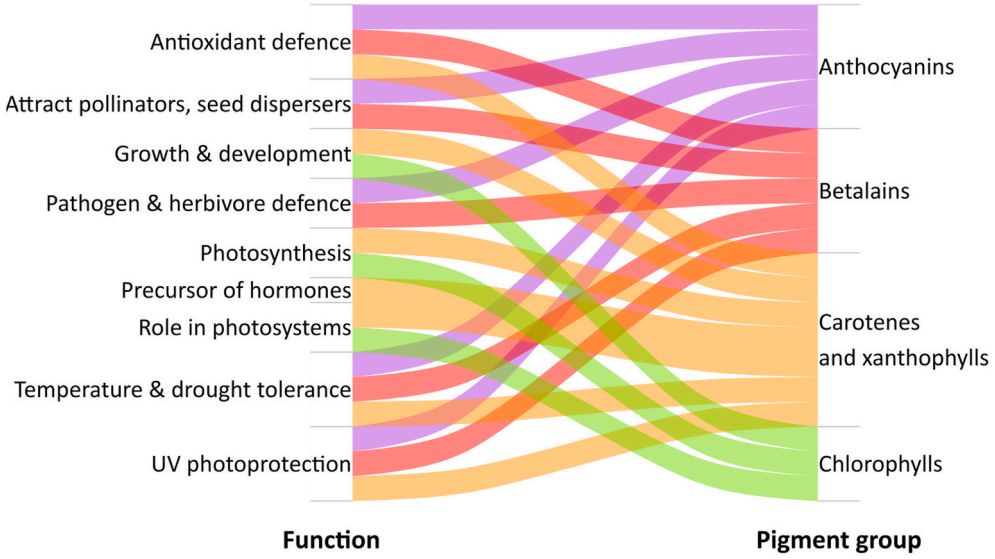

Fig. 3. Main functions of different reviewed natural pigment groups in plant cells. Compiled from Refs. [18–20].

excess. Another important aspect of anthocyanins in plants is the attraction of pollinators and seed dispersers [15,16]. The photoreceptors of different insects and pollinators allow these organisms to recognise flowers, thus ensuring the transfer of pollen between the plants. The attraction of pollinators is also determined by other groups of natural pigments [17]. Collectively, these functions underscore the ecological and physiological importance of anthocyanins as key components of the plant stress response and survival strategy (Fig. 3).

3.1.2. Chlorophylls

Chlorophylls are a tetrapyrrolic group of green plant pigments found in the chloroplasts of plants, algae, and cyanobacteria and are responsible for the absorption of red and blue wavelength radiation, converting the absorbed light energy into chemical energy through oxygenic photosynthesis. They are lipophilic molecules, which consist of a porphyrin ring structure with a central magnesium ion and a long hydrophobic phytol tail, which allows this molecule to bond to the lipid-rich membranes of chloroplast thylakoids. In 1772 chlorophyll's role was indirectly described by Sir Joseph Priestly, who noted that green plants absorb CO_2 and turn it into oxygen, a process later introduced as photosynthesis [21]. The term "chlorophyll" was first used by Pierre Joseph Pelletier and Joseph Bienaime Caventou at the beginning of the 19th century. Work on the elucidation of the molecular structure continued and at the beginning of the 20th century, German chemist Richard Willstätter first isolated pure chlorophyll and determined its structure, which, later, in 1915, granted him the Nobel Prize [21,22]. Chlorophyll *a* and chlorophyll *b* are the two most common types of chlorophylls. Chlorophyll *a* is the most abundant type of chlorophyll, followed by chlorophyll *b*. There are also distinct chlorophylls *c* and *d*, with specific absorption spectra, which are found in algae and cyanobacteria [23]. The different types of chlorophylls are distinguished by the variations in the side chains arising from the substitution pattern of the porphyrin ring [24]. An interesting example is chlorophyll *f*, which has a formyl group at the porphyrin ring, where chlorophyll *a* has a methyl group, thus altering the electron transfer and allowing absorption of far-red light (710-740 nm) [25]. The main functional role of chlorophylls is the absorption of light in the visible spectrum – chlorophyll *a* absorbs blue and red wavelengths, while chlorophyll *b* absorbs in blue-violet and red regions, thus also the visual appearance of both types of chlorophylls is slightly different. Since both of the mentioned chlorophylls absorb light at marginally different wavelengths, they also play a central role in photosynthetic adaptation. Depending on the light conditions in the environment, plants can optimise the energy capture by changing the ratio of chlorophyll *a* to b. In addition to the ability of chlorophylls to harvest the light and transfer the energy, they can also act as photoprotectants by dissipating the excess energy as heat, preventing oxidative damage to the photosynthetic mechanisms (Fig. 3). Chlorophyll metabolism can be affected by light intensity, temperature, and water availability. In drought and extreme temperatures, chlorophyll synthesis can be disrupted and the degradation is accelerated, thus affecting the photosynthesis as well as the phenotypical appearance of a plant [26]. Chlorophylls are mainly distributed within the plant's leaves, with chloroplasts abundant in the mesophyll cells to optimise maximum light absorbance [27]. Also, fruits, especially young fruits, can contain chlorophyll, which dissipates as the fruit ripens, with the exception of some species, for example, red grapes, which retain chlorophyll until they are ripe [28].

3.1.3. Carotenoids

Carotenoids (carotenes and xanthophylls) are two types of carotenoids naturally occurring as pigments in plants, algae, and some species of bacteria. Carotenes are a group of hydrocarbon carotenoids (containing only carbon and hydrogen atoms), while xanthophylls are oxygenated carotenoids (containing additional hydroxyl or epoxy functional groups), they share an isoprenoid structure. Carotenoids play an essential role in photosynthesis and photoprotection, as antioxidants and they are vitamin A precursors in humans [29]. The discovery of carotenoids dates back to the early 19th century when the orange-red pigment carotene was first isolated from carrots by Wackenroder in 1831. Soon after, an ethanol-soluble group of pigments – xanthophylls – was isolated from autumn leaves by Berzelius in 1837 [30]. The term "carotenoid" was coined only later and it wasn't until the early 20th century that the elucidation of carotenoid chemical structures happened. In 1901-1911, Mikhail Semyonovich Tswett, a Russian-Italian biologist, the inventor of chromatography, separated carotenoids for the first time, leading to their identification. M. Tswett started his research on chromatography on plant pigments and, interestingly, his surname, when translated from Russian, means "colour" [30,31]. The biosynthetic pathway of carotenoids was investigated throughout the 20th century, including the enzymatic aspects of the biosynthesis, including the formation of phytoene and its conversion into lycopene and cyclic carotenes, with the C40 skeleton being a key feature of these plant pigments [32,33]. Photoprotective ability of xanthophyll and the xanthophyll cycle was discovered in the 1960s, showing the protective effects of these pigments in photosynthetic damage prevention [34]. During the 20th century, the analytical instruments used for structural studies have evolved, thus leading to the identification of over 100 naturally occurring pigments [30,35]. Further developments in mass spectrometry and NMR allowed to determine the electronic properties of carotenoids, which were instrumental in understanding the different electronic and excited state properties of these pigments [36].

Carotenes and xanthophylls are pigments found in photosynthetic organisms like plants, algae, and certain species of bacteria. These compounds are synthesised in plastids, such as the chloroplasts, and localised in the thylakoid membranes of chloroplasts, where they play a critical role in light harvesting and energy transfer during photosynthesis [37,38]. In thylakoid membranes, carotenoids are bound to pigment-protein complexes, which are involved in light-harvesting complexes and are the reaction centres of photosystems [39]. Xanthophylls, for example, zeaxanthin and lutein, play an important role in the xanthophyll cycle, which helps in protecting the photosynthetic organism from light damage – they are the peripheral light-harvesting systems [40]. Xanthophylls can also be found in non-photosynthetic tissue, where their main role is as an antioxidant [37,41]. In non-photosynthetic tissues, such as flowers or fruits, carotenoids are stored in chromoplasts. In these organelles, the accumulation of carotenoids happens in lipid bodies or as crystalline structures, contributing to the pigmentation. Carotenoids function as accessory pigments in photosystem I and II, they absorb light in the 400-500 nm range, transferring the energy to chlorophyll *a,* thus helping in broadening the spectrum of light used for photosynthesis [37,42]. Carotenoids mitigate photooxidative stress by the quenching of reactive oxygen species, including the singlet oxygen generated during photosynthesis [38,39]. Xanthophylls participate in the non-photochemical quenching to dissipate the excess energy as heat [41]. The absence of dysfunctional biosynthesis of carotenoids has shown to introduce light stress and oxidative damage, for example, in mutants lacking zeaxanthin and lutein in *Chlamydomonas reinhardtii*, which has been shown to create irreversible photooxidative bleaching and cell death under elevated light conditions [43]. Carotenoids are essential for the physiological function and survival of photosynthetic organisms; their involvement in light harvesting, photoprotection, antioxidant activity, and pigmentation shows their critical role in cellular homeostasis and activity against abiotic stressors (Fig. 3). The wide distribution and functional diversity of carotenoids underscore the evolutionary significance and adaptation to different environmental contexts.

3.1.4. Betalains

Betalains are a group of alkaloid pigments that are found in 13 specific families of Caryophyllales [44]. Historically, betalains have been named "nitrogenous anthocyanins" [45], which implies the

similarities between betalains and anthocyanins, also considering the specifics of pigment location within the plant tissue. Structural characteristics of betalains were discovered only later, and the nitrogen-containing molecule was named after the betalamic acid, which is found in red beetroot (*Beta vulgaris*) [46]. The betalain group consists of two main pigment classes, the orange-yellow pigment betaxanthin and the red-violet betacyanin [47]. The main betalain substructure is protonated 1,2,4,7,7-pentasubstituted 1,7-diazaheptamethin, which is the key component forming the chromophore of betalains responsible for light absorption and colour characteristics [48].

In plants, several factors can influence the location of betalain biosynthesis. Depending on the growth stage, betalains can be present in the aboveground parts of the plant (leaves, stems, flowers, fruits, or seeds) as well as in the belowground parts, the roots, as in the case of the red and orange beet roots [49]. Distribution of betalains is species dependent; for example, the Cactaceae family contains betalains strictly in the fruit, while betalains in beetroot are present in the root as well as in the veins on the leaves [50]. Betalains are also present in some higher fungi, *Amanita*, *Hygrocybe*, and *Hygrosporus* [47]. The distribution and the form of storage within the plant of betalains are somewhat similar to anthocyanins; both are stored in the cell vacuoles as the respective glycosides [51]. Despite the similarities in the colour, response to environmental stressors and cellular localisation, these two pigment classes are considered mutually exclusive, there are no known plant species that naturally biosynthesize both betalains and anthocyanins [52]. Biosynthetic pathways of betalains involve different enzyme systems than those utilised in anthocyanin synthesis; moreover, the precursor of betalain biosynthesis is tyrosine, while anthocyanins are synthesised from phenylalanine through the flavonoid pathway [53,54]. Phylogenetic studies suggest that betalain synthesis has been present in several species in the past, and through evolution, these species have switched to anthocyanin synthesis instead, while keeping some betalain synthesis-relevant homologous enzymes that can also glycosylate anthocyanidins [52,55]. Interestingly, some species in the betalain-synthesising family Caryophyllales have switched back to anthocyanin synthesis [52]. This genetic exclusivity of the biosynthesis of betalains and anthocyanidins indicates that the role of anthocyanins is substituted by betalains in certain species or perhaps betalains offer some specific adaptation traits to certain environments that anthocyanins do not. Functionality of betalains within the plant is also shared with the function of anthocyanins. The main roles of betalains are: the ability to scavenge reactive oxygen species and act as antioxidants, attraction of pollinators [56], regulation of osmotic pressure, response to changes of light conditions within the environment and UV irradiation (photoprotectants) [57], as well as abiotic stress factors such as low temperature (Fig. 3), dry conditions, and salinity caused stress (especially in arid areas) [58–60]. Despite the similarities in pigment functions between anthocyanidins and betalains, betalains lack certain functions that anthocyanidins have, for example, the ability to increase stress tolerance against certain metals and the ability to deter herbivores [59].

3.2. Chromatic properties and stability of natural plant pigments

3.2.1. Anthocyanins

The core of an anthocyanin molecule, the chromophore, is the flavylium cation (2-phenylbenzopyrylium with a positive charge), which has a delocalised π electron system with conjugated double bonds that absorb visible light [61]. Due to this conjugated molecular structure across the aromatic rings, resonance within the molecule is possible; the energy gap is lowered between the highest occupied and lowest unoccupied orbitals, also known as the HOMO-LUMO gap, giving the ability to absorb the visible light within the range of 490 to 540 nm [62]. Another aspect contributing to the chromatic properties is the number of substitutions; more hydroxylation leads to an increase in absorption wavelength (towards the blue colour), while a higher number of methoxylations decreases the absorption wavelength (towards the red colour) [63]. Similarly, glycosylation and acylation can lead to slight changes in the hue of anthocyanins, as well as changing their solubility and stability. Arguably, one of the most important factors in anthocyanin chromatic properties and their stability is the effect of different pH on the molecule [64]. Anthocyanin chromophore contains a strong electron-withdrawing pyrillium ring, giving the molecule acidic properties; they can be considered weak diacids. With pH increase, the anthocyanin molecule becomes deprotonated, thus increasing the electron polarisation and changing the light absorption to higher wavelengths. Hydroxyl groups attached to the chromophore have differing pKa values (acidity); the hydroxyl group at C7 has the highest pKa value, followed by C4 and C5. Depending on the pH of the environment, the equilibrium between these hydroxyl groups determines the colour of the anthocyanin (Fig. 4). At pH 1-3 the anthocyanin chromophore is fully protonated and has a positive charge, creating molecular species called the flavylium cation (red colour) [65]. At pH of about 3.5-6 the equilibrium created leads to the flavylium ion shifts. Hydration of C2 occurs, leading to the opening of the ring and rearrangement into a hemiketal structure. Since the aromatic ring system is disrupted, the delocalisation of electrons does not occur, resulting in the elimination of light absorption and loss of colour in the visible spectrum. pH increase to 6-7 causes deprotonation, converting the flavylium cation into a neutral quinonoid base with purple colour [65]. Further increase of pH creates an anionic quinonoid base with blue to blue-greenish colour. If pH is increased even further (pH $>$ 8), a chalcone (open form) is created – this causes the ring opening and permanent loss of colour due to it. The exact pH intervals at which each of the shifts occurs depend on the type of anthocyanidin as well as the structure, whether the molecule is glycosylated, methylated, or acylated. The anthocyanins' ability to change the colour depending on the pH is one of the most important aspects and limitations of their application [66].

3.2.2. Chlorophylls

Chlorophylls are made up of a cyclic tetrapyrrole macrocycle, called porphyrin, which consists of four pyrrole units linked by methine bridges creating a conjugated π-system. In the centre of the porphyrin ring is a magnesium ion which stabilises the molecule and electron transfer [67]. The role of magnesium ion in the porphyrin system is to lower the energy gap between the HOMO-LUMO, thus enabling the absorption of visible light. The porphyrin ring has a planar structure, which allows for extensive conjugation ensuring strong visible light absorption capabilities [68]. The absorption spectrum of chlorophylls is characterised by absorption maximum at two visible light regions – blue-violet light (Soret band) and red region (Q band), which are ensured by the delocalised π electrons within the molecule. Substitutions on the porphyrin ring can influence the light absorption properties of the molecule, for example, chlorophyll *a* has a methyl group at position 7, while chlorophyll *b* has a formyl group, shifting the absorption towards shorter wavelengths [68]. Magnesium ion can also alter the electron transitions, thus affecting the absorption spectrum by substituting the magnesium ion with, for example, copper, the absorption spectrum is shifted, which also creates a more stable molecule – copper chlorophyllin. Chlorophyll appears green due to the molecules ability to effectively reflect the green light (Fig. 5), while absorbing the complementary red and blue light through a process known as subtractive colour mixing [69].

Within the cell, chlorophylls are stabilised by binding to photosynthetic proteins within the chloroplast's thylakoid membrane, however, they can be degraded through the PAO/phyllobilin pathway by several enzymes that removes the phytol tail, magnesium ion, and open the porphyrin cycle [20,70]. Extrinsic factors like elevated temperatures, oxygen, pH, metal ions, and exposure to light can also influence the stability of chlorophyll [71]. High intensity of light can induce degradation by generating reactive oxygen species that damage the molecule.

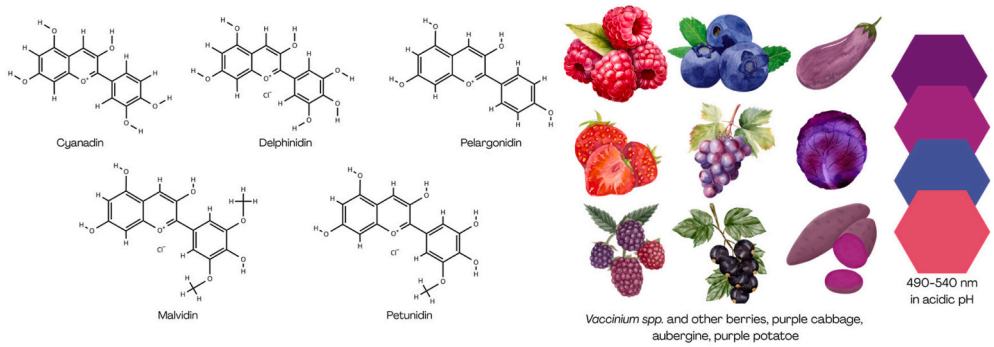

Fig. 4. Major types of anthocyanidin backbones most commonly found in nature, common food sources and associated colours with indicative reflection wavelengths. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

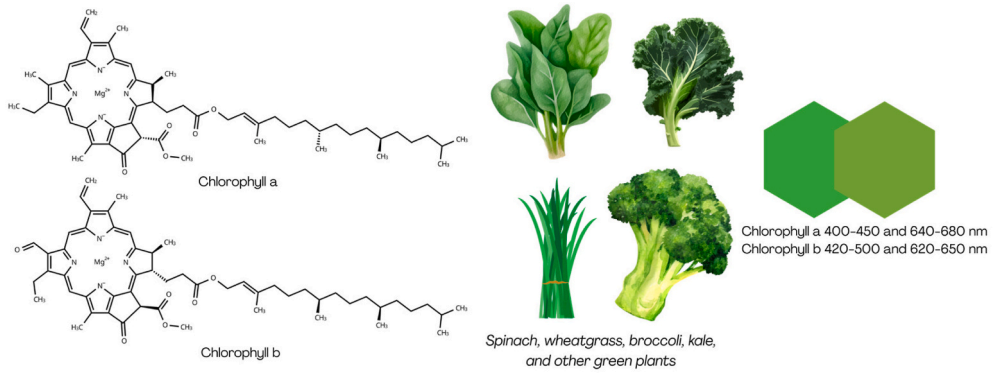

Fig. 5. Major types of chlorophylls, common food sources and associated colours with indicative reflection wavelengths. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

High temperatures can influence the degradation of chlorophyll by denaturing the photosynthetic proteins the chlorophyll molecules are bound to, thus leaving them exposed [72]. Also, pH can affect chlorophyll stability – low pH can promote the removal of the magnesium ion, which leads to the degradation and formation of the chlorophyll degradation product pheophytin [73]. Chlorophylls are ubiquitously distributed across photosynthetic organisms, with particularly high concentrations found in green leafy vegetables and fast-growing photosynthetic tissues (Fig. 5). Major dietary and industrial plant sources include spinach (*Spinacia oleracea*), kale (*Brassica oleracea*), parsley (*Petroselinum crispum*), broccoli (*Brassica oleracea* var. italica), and cereal grasses such as wheatgrass and barley grass [24,69]. In addition to higher plants, microalgae and cyanobacteria, including *Chlorella* spp. and *Arthrospira* spp., represent highly concentrated and scalable sources of chlorophyll, which are increasingly exploited for food, nutraceutical, and functional ingredient applications. The relative abundance of chlorophyll *a* and *b* varies among species and tissues, reflecting adaptation to light conditions and photosynthetic efficiency [74].

3.2.3. Carotenoids

The coloration of carotenoids originates from the chemical structure of their chromophore – it is a system of conjugated double bonds arranged in an alternating pattern of double-single bonds in a linear or cyclic carbon backbone [75]. The arrangement of single and double bonds influences the delocalisation of π-electrons and electronic transitions, thus changing the absorption spectrum [75]. The conjugated polyene chain is what allows the molecule to absorb the light in the visible range, variations in this structure are directly related to the versatility of carotenoid colours observed in nature [76,77]. The length of the conjugated system is the primary determinant of the carotenoid light absorption properties: as the number of conjugated double bonds increases, the molecule absorbs longer wavelengths, resulting in a red shift and deeper coloration [78]. On the other hand, structural features like a higher number of single bonds (Fig. 6) can cause rotation in the polyene chain, which reduces the effective conjugation length and rotation around single bonds and leads to absorption of lower wavelengths (lighter colours) [79]. In addition to the double-single bond placement and chain length, the presence of methyl, hydroxyl, and keto groups can alter the light absorption significantly [80]. Depending on the electronic properties of the substitutions and the positions along the polyene chain, they can either act as electron donors or acceptors, thus altering the energy levels involved in light absorption. For example, the hydroxyl groups in zeaxanthin lead to a blue shift, while a keto group present in astaxanthin shifts the absorption maximum to longer wavelengths (red shift) [81]. Carotenoids can also be cyclic, for example, *β*-carotene, containing a ring structure that limits the conformational flexibility and influences the distribution of π-electrons along the chain. A more rigid structure, like that of *β*-carotene, has distinct absorption properties compared to the acyclic counterparts [82]. Beyond the molecular conformation that influences the light absorption of these molecules, extrinsic factors can also influence the pigmentation properties of these molecules. Carotenoids can interact with proteins or other molecules, which changes the polarity, hydrogen bonding, and steric constraints, thus changing the energy shift and electron transitions, altering the visible colour without changes in the molecular structure of the carotenoid itself [75,79].

Carotenoids are generally highly reactive due to their extended conjugation of double bonds, which are prone to oxidation and isomerisation. These changes lead to a reduction of colour intensity as well as a decline in nutritional value [83]. Primarily, carotenoids can be found in all-trans configuration, which is thermodynamically favoured

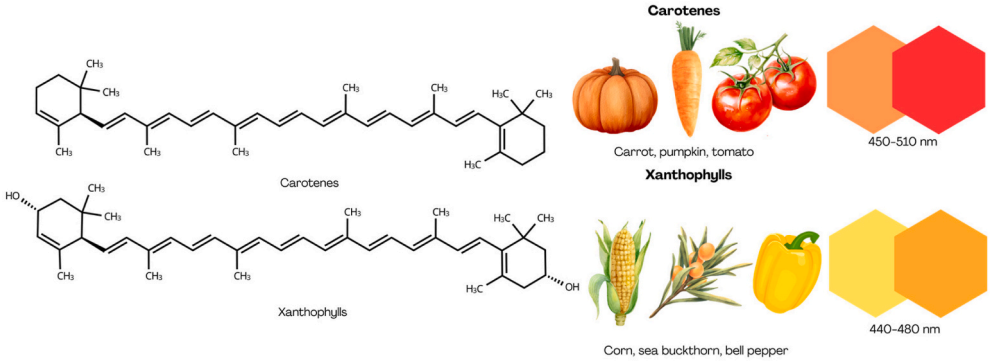

Fig. 6. Major types of carotenoids, common food sources and associated colours with indicative reflection wavelengths. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and has higher bioactivity, however, when exposed to heat, light, or chemical stressors, conversion to cis-isomers happens, which reduces the bioavailability and alters the absorption characteristics [84]. While carotenoids are known as antioxidants under certain oxidative conditions or other redox-active components, they can act as prooxidants, leading to accelerated lipid peroxidation and degradation [85]. This complex behaviour adds further challenges in carotenoid stabilisation and their incorporation in formulations. Environmental factors such as light, heat, and oxygen play a critical role in carotenoid stability. Elevated temperatures can accelerate both oxidative degradation and isomerisation. Exposure to light can induce photo-oxidation, thus products enriched with carotenoids should be processed, packaged, and stored in dark conditions. Oxidation of carotenes can also be avoided by vacuum or inert-gas packaging to enhance the shelf-life of the products they contain [86]. Neutral pH is optimal for carotenoid stability, while both highly acidic and alkaline environments promote degradation. Certain metal ions can either stabilise or degrade carotenoids, depending on the specific redox interactions involved [87]. The complex structure and number of double bonds are not favourable for stability under varying environmental conditions, the complex interplay of intrinsic molecular features and extrinsic environmental conditions must be carefully considered when creating stabilisation techniques and further formulations for incorporation into food matrices [72].

3.2.4. *Betalains*

Betalain molecules contain a system of conjugated double bonds, which allow for the delocalisation of electrons through the structure, lowering the energy required for electron transition, thus giving the molecule the ability to absorb light within the visible range [88]. Colour specificity in betalain molecules is altered through additional structural modifications – acylation, glycosylation. Betaxanthins are fluorescent with emission maximum in the range of 508–608 nm and fluorescence may be of importance in the signalling between flowers and pollinators [89]. Betalain fluorescence has found a variety of interesting applications, supporting high sensitivity of its detection in plant and animal tissues [89]. Betalains can be used in biosensors, for example, for analysis of copper ions [90], to monitor ingestion and digestion of betalains, for labelling of proteins [91], and in many other applications [47]. Carboxylation of the betalain molecules can increase the fluorescence of betaxanthin [92]. Betacyanins, the red to violet betalains, absorb visible light at 532 – 541 nm, they contain betalamic acid condensed to L-3,4-dihydroxy-phenylalanine (cyclo-DOPA) or its glucosyl derivatives [93]. Other functional groups within betacyanins, for example, hydroxyl groups, interact with the conjugated system, thus altering the absorption wavelengths. Betaxanthins, the yellow to orange betalains, absorb the visible light at 458 – 485 nm, they also consist of the basic betalamic acid, however, betaxanthins are condensed with amino acids, for example, tryptophan, phenylalanine, or derivatives (Fig. 7, [93]). The specific interaction between the amino acids and the functional groups alters the visible light absorption, contributing to the range of betaxanthin hues.

Betalain stability depends on its structure and can be influenced by pH, water activity, oxygen and oxidising agents, light, metal ions, temperature, and other factors [93–96]. A key factor affecting their stability is their molecular structure, protection of more labile structures is targeted primarily [47]. Esterification with aromatic acids [97], esterification with aliphatic acids, glycosylation [98] can increase betalain stability. The colour of betalains can also be influenced by pH due to protonation/deprotonation of certain functional groups. Lower pH shifts the absorption wavelength to lower values, while more

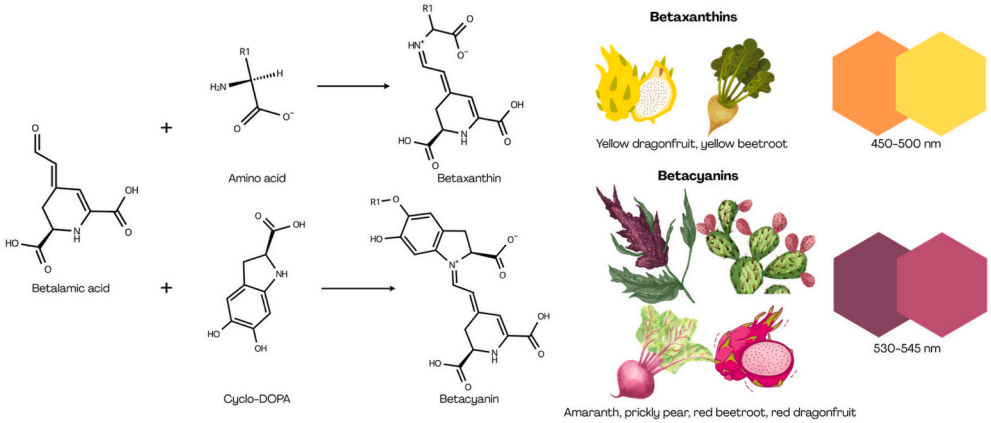

Fig. 7. Simplified betaxanthin and betacyanin biosynthesis, common food sources, and associated colours with indicative reflection wavelengths. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

alkaline pH increases the absorption wavelength [51]. However, compared to anthocyanins, betalains are more pH stable, the pH range at which betalains can be considered stable is pH 3-7, while anthocyanins change their colour in pH higher than 3, meaning that betalains are more suitable for a wider range of products, especially products with neutral or slightly acidic pH [48,51]. UV and visible light radiation excites π electrons and thus the reactivity of betalains and the effects depend on the energy of irradiation. The following betalain degradation depends on the presence of oxidants (at first, oxygen) [50]. Metal ions reduce betalain stability but if complex-forming substances (e.g., citric acid, EDTA, etc.) are added, molecules are stabilised ([99]; Sen Arslan and Altınok 2025).

4. Recovery of natural pigments from food industry by-products

4.1. Anthocyanins

Anthocyanins represent an important class of natural pigments found in flowers, fruits, and vegetables, ranging from red to blue. Besides their pigment properties, they also possess different beneficial properties, such as antioxidant, antimutagenic, and anti-neurodegenerative effects contributing to the overall human health and disease prevention [100–102]. Generally, a critical aspect of sustainable resource management is the valorisation of food waste. Fruit processing industries globally generate substantial amounts of biowaste, often accounting for 10% to 60% of the initial raw material weight [103–105]. This biowaste, particularly from berries, is rich in valuable bioactive compounds, including anthocyanins, phenolic acids, and other flavonoids [104,106]. The recovery of these phytochemicals from agricultural and food processing by-products is a key strategy for enhancing resource-use efficiency, promoting circularity, and developing new, sustainable products such as functional foods, supplements, and pharmaceutical or cosmetic ingredients [104,107,108]. The utilisation of these low-cost by-products offers a promising avenue for environmentally conscious and economically viable production of high-value-added compounds [102,109].

Conventional solvent extraction (CSE) is the most commonly used method for anthocyanin extraction from plant material [110–112]. Due to the water-soluble nature of anthocyanins, polar solvents are generally preferred (Table 1). To enhance stability and the extraction yield, considering the anthocyanin instability in alkaline solutions, acidified aqueous solvent systems are typically employed. Most commonly used solvents are acidified water, ethanol, methanol, or their mixtures [100,102,105]. An ethanol-hydrochloric acid-water mixture (60%:0.1%:39.9% v/v/v) has been used to extract purple corncob anthocyanins [112], acidified ethanol (1% hydrochloric acid) for jabuticaba peel [113], while raspberry pomace has been extracted using 80% methanol [109]. Optimisation of CSE often employs statistical tools like Response Surface Methodology (RSM) and Central Composite Design (CCD) to evaluate the influence and interactions of multiple factors, aiming to maximize extraction yields while minimising resource consumption [101,102]. CSE is a relatively simple, inexpensive, and easy-to-perform method [101,109]. The use of ethanol is advantageous as a generally recognised as safe (GRAS) solvent permitted for food applications [101]. However, CSE can be time-consuming and less efficient if compared to ultrasound-assisted extraction or other intensive extraction methods [112].

Ultrasound-assisted extraction (UAE) leverages the high-frequency sound waves to induce cavitation within the solvent, which facilitates cell wall disruption, increasing solubility, diffusion, and solvent penetration, thus accelerating the transport of compounds of interest from the plant matrix to the solvent [105]. Ultrasound-assisted extraction has been widely used for berry pomace and residue extraction. Extraction of blackcurrant pomace has been studied, where parameters such as temperature (25, 35, 45 °C), time (15, 30, 60 min), and material/solvent ratio (1:20 or 1:7) were studied using acidified water-ethanol or water-citric acid solvents [105]. Multi-frequency UAE has been investigated for blueberry pomace, showing promising results by combining frequencies to enhance anthocyanin yields [116]. Optimal conditions for multi-frequency UAE of blueberry pomace were found to be 40 kHz followed by 80 kHz, 350 W power, 50 °C, and 40 min extraction time [116]. UAE is a fast and efficient method, when compared to conventional extraction techniques, leading to reduced extraction time, energy consumption and solvent usage [105,109]. While generally efficient, excessive exposure to ultrasonic waves can lead to the degradation of anthocyanins, potentially resulting in lower or comparable yields to conventional methods in some cases [105,113,116]. For example, the UAE was found to be less effective for total polyphenolic (TPC) and total anthocyanin (TA) extraction from jabuticaba peel compared to CSE under certain conditions [113].

Cold Plasma-Assisted Extraction (CPAE) is a relatively novel technique that utilises high-energy plasma (e.g., helium plasma) to effectively break down the lignocellulosic structure of plant cell walls, thereby facilitating the rapid release of intracellular compounds [112]. It has been developed for the extraction of anthocyanins from purple corncob, with optimised conditions including a discharge power of 127 W, working pressure of 150 Pa, and a treatment time of 75 s [112].

Table 1
Anthocyanin extraction from various agri-food wastes.

Source of anthocyanins	Extraction conditions	Extraction solvent	Yield of anthocyanins	Reference
Hibiscus sabdariffa calyxes	Ultrasound-assisted extraction for 30 min	Acidified water/ethanol (20:80 v/v) solution	Delphinidin 3-O-sambubioside 15%	[100]
Zea Mays purple corncob biomass waste	Conventional solvent extraction at 50 °C for 2 h; Cold plasma assisted extraction for 20-120s	Ethanol/hydrochloric acid/water (60:0.1:39.9%); Helium cold plasma	Cyanidin-3-O-glucoside (25.95%); Petunidin-3-O-glucoside (0.02%); Pelargonidin-3-O-glucoside (66.02%)	[112]
Rubus idaeus fruit pomace	Ultrasound assisted extraction for 120 min at 50 °C; maceration for 120 min at 20 °C	80% methanol acidified with 1% formic acid	Cyanidin-3-glucoside (7.13 mg/L) as total anthocyanin content	[109]
Ribes nigrum fruit pomace	Ultrasound-assisted extraction at 35 °C for 15 min	Water–ethanol solvent (50/50 v/v) acidified with HCl	Total anthocyanin contents 277 mg/100g	[105]
Sabará jabuticabas	Ultrasound-assisted extraction for up to 30 min, peel/solvent ratio 1:10	96% ethanol acidified with 1% HCl	Total anthocyanins up to 484.65 mg/L	[113]
Aronia melanocarpa fruit pomace	Ultrasound-assisted extraction 20 min	1.5 wt% citric acid in water, 45 °C and 34 g solvent/g pomace ratio.	Total anthocyanins 456.7 mg/L	[114]
Vaccinium corymbosum fruit pomace	Maceration for 100 min at different temperatures 30-90 35 °C	Acidified water and 80% ethanol to pH 2.0	Total anthocyanins 2045 μg/g	[115]
Eugenia involucrata biowaste	Maceration at room temperature	80:20 ethanol:water, acidified with citric acid to pH 3	Total anthocyanins 12.0 mg/g	[104]
Malpighia emarginata fruit waste	Maceration for 2-90 min at 20-90 °C	Ethanol at different concentrations (0-96%) with 0.05% citric acid	Total anthocyanins 2.54 mg/g	[101]
Allium cepa red onion skin waste	Conventional extraction shaking at 150 rpm for up to 4h at 16-60 °C	Ethanol acidified with citric acid in different ratios	Total anthocyanins from 0.45 to 1.43 mg/g	[102]

Representing a highly efficient and green process, CPAE offers advantages such as low cost, short treatment time, and scalability for industrial production [112]. CPAE has demonstrated significantly higher anthocyanin yields compared to both CSE and UAE by effectively breaking down the protective lignocellulosic structures in plant cell walls [112]. Considering the sustainability goals and aims for advanced biomass utilisation, sequential extraction approaches have been studied to recover multiple compounds from the same raw material in successive steps, thus maximising the utilisation of the biomass. A notable application involves the sequential extraction of anthocyanins and pectin from jabuticaba peel [113].

A common drawback across different extraction methods is that temperatures exceeding certain thresholds (e.g., 60 °C) can cause degradation of anthocyanins, leading to a loss in yield and quality [101,102,115]. The high moisture content and residual carbohydrates of fresh pomace make it highly susceptible to microbial spoilage, necessitating rapid processing or pre-treatment [105]. While necessary for storage and processing, methods like drying can lead to thermal degradation of compounds, and freezing can cause cellular damage and loss of natural pigment in the plant material [113]. The findings consistently highlight the promising potential of food waste as a resource for developing innovative products (Table 1). This includes applications in therapeutic medicines, pharmaceuticals (particularly targeting abnormal cell growth due to their anti-proliferative effects), natural preservatives, and eco-friendly natural colorants for various industries like food and textiles [104]. The continued focus on economically efficient extraction is crucial for successful industrial implementation, especially given the low cost of the raw biowaste materials [102]. The development of green extraction methods and modelling techniques, such as those using artificial neural networks, will serve as important references for the food industry in utilising pomace efficiently [115].

4.2. Chlorophylls

Chlorophylls have emerged as promising candidates for the food industry due to their desirable colouring properties as well as the bioactive properties (antioxidant, antibacterial, and UV protective activities), giving extra functionality to the products using these natural pigments as additives [117–119]. Strategic valorisation of various side-streams can stimulate sustainable economic growth and mitigate ecological impacts caused by improper disposal and underutilisation of certain types of biomass [120,121]. Despite the promising nature of chlorophylls, their wider acceptance and application are hindered by their intrinsic instability due to environmental factors, indicating the need for new and innovative extraction and stabilisation techniques [117,118,122].

Several methods have been investigated to retrieve chlorophyll from food and agro-wastes, frequently incorporating principles of green chemistry to increase the sustainability of the process and improve extraction yields (Table 2). Microwave-assisted extraction (MAE) has been used to derive natural chlorophylls from various types of wastes. Chlorophyll extraction from spinach waste has been done using deionised water, followed by extraction in ethanol at 100 W for a duration of 10 min, maintaining a temperature of approximately 55 °C [118]. Similarly, chlorophyll was successfully extracted from broccoli waste utilising MAE, using 90% (v/v) acetone at a liquid to solid ratio of 5:1, heated at 6 W per gram for 25 min, consistently holding a boiling temperature around 60 °C [122]. Investigation of MAE has continued due to its speed, cost-effectiveness, and environmental benefits – this method facilitates the rupture of plant cell walls, therefore opening up the cells and allowing for diffusion to take place, which increases the overall extraction yields, when compared to conventional extraction techniques [118,122]. However, MAE has the potential to alter the chemical structure of chlorophyll and the chlorophyll-cell wall material interaction. Since MAE is a high-intensity extraction method where the release of large amounts of energy is happening, there is a possibility of chlorophyll degradation due to excess heat [118].

Table 2
Chlorophyll extraction from various agri-food wastes.

Source of chlorophylls	Extraction conditions	Extraction solvent	Yield of chlorophyll	Reference
Beta vulgaris stems and leaves	Conventional extraction at 20 °C, solid: liquid ratio 0.12 for 70 min	Quaternary ammonium-based ionic liquid (N-ethyl-N-methyl-N,N-bis(2-hydroxyethyl) bromide) and polypropylene glycol with water	Total chlorophylls 1.82 wt%	[123]
Pisum sativum pod waste	Ultrasound-assisted extraction for 27.7 min at 33 kHz, 46.05 h soaking time	92.85% ethanol	Total chlorophylls 0.642 mg/g	[119]
Actinidia deliciosa peels	Ultrasound-assisted extraction at 55 and 60 °C for up to 30 min	100% methanol	Total chlorophylls 317.89 μg/g	[124]
Arthrospira platensis extraction residue	Ultrasound-assisted extraction for 1 h at 50 °C, pH 6, S/L ratio 1:8	100% ethanol	Total chlorophylls 5.96 mg/g	[120]
Spinacia oleracea waste	Conventional extraction for 30 min at 60 °C and microwave-assisted extraction for 10 min at 55 °C	96% ethanol	Chlorophyll *a* 5.74 mg/g Chlorophyll *b* 3.89 mg/g	[118]
Brassica oleracea var. italica wastes	Microwave-assisted extraction for 25 min at 60 °C	90% acetone	0.2 g chlorophyll loaded in 3 g hybrid nanopigments with absorption values up to 98.44%	[122]
Ipomoea batatas leaves	Conventional leaching extraction, S/L ratio 1:10, for 100 min at 90 °C, pH 6.5	96% ethanol with water in a ratio 4:6	Chlorophyll extract used directly for application as a functional fabric dye	[117]
Mangifera indica cv. Ataulfo waste	Conventional shaking extraction for 2 h	100% acetone	Total chlorophyll in seed oil – 0.902 mg/kg; in peel flour 457 mg/kg	[121]

UAE, widely used for biomass extraction, has also been used for chlorophyll extraction. Pea pod waste extraction has been optimised using 92.85% ethanol, a sonication time of 27.7 min, and a soaking period of 46.05 h, at a solid-to-solvent ratio of 1:10 (w/v), with temperatures exhibiting a rise from 25 °C to 36 °C during the sonication phase [119]. UAE is recognised as a green technology that induces acoustic and hydrodynamic cavitation, enhancing solvent penetration into the sample and accelerating mass transfer rates, which consequently increases pigment yield [119]. This method has also demonstrated superior efficacy in sterilisation compared to thermal treatments

alone, as shown in studies involving kiwi peel, where thermosonication (ultrasound coupled with mild heat) led to effective bacterial inactivation while retaining key nutrients and bioactive compounds [124]. Similarly to MAE, UAE can also generate excessive heat, which leads to degradation of the pigments, especially in cases when the temperature as well as the sonication time have not been controlled [124].

Chlorophylls have also been extracted using CSE from *Ipomoea batatas* leaves through a solvent leaching method utilising an optimised water/ethanol ratio of 4:6, a mass-to-liquid ratio of 1:10 g/mL, at 90 °C for 90 min, and a pH of 7 [117]. Similarly, chlorophylls were effectively recovered from the spent biomass of *Arthrospira platensis* (residual biomass after phycobiliprotein extraction) using 100% ethanol at a 1:8 solid-liquid ratio, pH 6, 50 °C, and a 1-h extraction time [120]. Chlorophyll from spinach was also conventionally extracted with ethanol at 60 °C for 30 min, followed by chilling and centrifugation to separate the protein fraction [118]. The principal advantage of this method resides in its straightforwardness and well-established protocols, rendering it accessible and economically feasible [117]. However, conventional solvent extraction can be time-intensive, frequently demanding multiple cycles, and the extended exposure of pigments to environmental elements such as light, oxygen, and high temperatures (if heated to improve mass transfer) can reduce their stability and lead to degradation [117,120].

A thermoreversible aqueous biphasic system was developed for the extraction and selective separation of both betalains and chlorophylls from red beet stems and leaves. The process employed a monophasic aqueous solution comprising 10 wt% of the ionic liquid, 52% polypropyleneglycol (PPG), and 38% water, with pigment extraction conducted at 20 °C for 70 min using a solid:liquid ratio of 0.12, followed by phase separation induced by elevating the temperature to 35 °C [123]. An important benefit of this method is its capacity for one-step extraction and selective separation of multiple pigments into distinct phases, alongside the utilisation of carefully designed ionic liquids that can be non-toxic and biodegradable [123]. Moreover, these ionic liquids have been shown to effectively stabilise pigments like betalains, and the process facilitates high pigment recovery and the potential for ionic liquid reuse [123]. Valorisation of the waste streams into natural pigments and functional materials aligns with the circular economy paradigm, holding potential to reduce pollution and improve the sustainability of the food production sector and other industries (Table 2).

4.3. Carotenoids

Increase of food processing has led to the generation of agricultural and food industry waste, these by-products include peels, pomace, and seeds from fruits and vegetables and they are recognised as valuable, low-cost, and relatively abundant sources of various phytochemicals, particularly carotenoids (Table 3). Carotenoids have widespread applications in the food, pharmaceutical, and cosmetic industries due to their vibrant colour, antioxidant activity, anti-inflammatory, and immunity-enhancing properties [125,126]. Carotenoids have also been reported to have a role in cancer prevention, cardiovascular disease, and various age-related disorder prevention [127–129]. Traditional extraction methods for recovery of carotenoids involve high solvent consumption, prolonged exposure times, low extraction yields, and are a concern regarding the environmental impact of the process [130]. To counter the negative aspects of carotenoid extraction, there is an incentive to develop and optimise green extraction methods that would be more efficient, cost-effective, and environmentally friendly, thus ensuring the safety and quality of the extracted compounds [129,131].

Recent research has explored various extraction processes to recover carotenoids from food waste, focusing on enhancing yield, purity, and sustainability. Conventional extraction methods (CE), such as maceration and Soxhlet extraction, typically involve prolonged exposure between the plant biomass and the used organic solvent, most often n-hexane, ethanol, ethyl acetate, or acetone (Table 3). CE has been used, for example, for the extraction of passion fruit peel using olive oil and tomato processing waste using acetone [125,133]. Depending on the solvent used, the concentration of carotenoids in the final extract can differ and the processing of the created extract can be hindered due to the presence of non-volatile solvents like oil. While CE methods are simple and require minimal equipment, they are suitable mostly for the production of crude extracts – the solvents used for carotenoid extraction can solubilise a variety of other plant metabolites, thus creating complex raw extracts [126]. Moreover, the processing times and solvent consumption of CEs are generally high. The recovery of solvents from the extract usually requires heating of the sample and thus the carotenoids are often degraded during the concentration and clean-up process [125,128].

Ultrasound-assisted extraction has been used to extract carotenoids from passion fruit peel, habanero chili pepper waste, pumpkin peel, and orange peels using various solvents, including vegetable oils and hydrophobic deep eutectic solvents [125–127,129]. The time required for extraction is reduced when using UAE compared to CE, also the yields reported are generally higher and the solvent consumption is reduced. On an industrial scale, UAE usage can be considered a more cost-effective alternative to CE [125,127]. An aspect to be considered when using UAE is the thermal degradation induced by the heating effect of high-frequency ultrasound and the possible isomerisation of

Table 3
Carotenoid extraction from various agri-food wastes.

Source of carotenoids	Extraction conditions	Extraction solvent	Yield of carotenoids	Reference
Citrus sinensis peels	Ultrasound assisted extraction at 45 °C for 20 min, S/L ratio 1:20	Hydrophobic deep eutectic solvent (HDES) – menthol:eucalyptol	Total carotenoids 573.4 mg/100 g	[127]
Citrus sinensis peels	Conventional stirring extraction for 60 min at 500 rpm, S/L ratio 1:10	HDES – thymol:hexanoic acid (2:1)	Total carotenoids 259 μg/g	[130]
Passiflora edulis peels	Ultrasound-assisted extraction for 50 min at 50 °C, S/L ratio 1:21-30	Vegetable oils – olive and sunflower oils	Total carotenoids 1240 and 1176 μg/100 g in olive and sunflower oil, respectively	[125]
Cucumis metuliferus Mey. Ex. Naudin peels	Cloud point extraction for 43 min at 55 °C, pH 7.32, S/L ratio 1:70	Distilled water with 10% Tween 80	*β*-carotene 13.11 mg/100 g	[132]
Cucurbita maxima peels	Ultrasound assisted extraction at 80 °C, for 100 min, S/L ratio 1:10	n-hexane/acetone solvent mixture 3:1	Total carotenoids 1.06 mg/g	[129]
Capsicum chinense (red habanero) waste	Several methods evaluated – maceration, ultrasound, Soxhlet, SFE, SFE + co-solvent (best yield)	Supercritical CO_2 with ethanol as co-solvent	Total carotenoids 292.09 μg/g	[126]
Prunus persica pomace	Ultrasound-assisted enzymatic extraction for 50.36 min at 45.18 °C	Pectinase solution 8.5%	Total carotenoids 0.13 mg/L	[128]
Daucus carota subsp. Sativus sorting waste	Microwave-assisted extraction for 5 min at 50 °C, S/L ratio 1:40	Hexane:ethanol 1:3	Total carotenoids 192.81 mg/100 g	[131]
Solanum lycopersicum processing waste	Conventional stirring extraction for 90 min at 50 °C	100% acetone	Total carotenoids 28.86 mg/100 g	[133]

carotenoids due to the processing [125,127]. UAE can also be combined with the catalytic action of enzymes to help in the breakdown of plant cell walls, thus enhancing the release and extraction of carotenoids [128]. The combined effects increase the extraction yield, however, the processing time and costs are also increased [128].

Microwave-assisted extraction (MAE) leverages microwave energy to induce dipolar rotation and ionic conduction within the plant matrix and solvent, generating internal heat that weakens and ruptures cell walls, facilitating the release of phytochemicals into the solvent [125]. This technique has been investigated for carotenoid extraction from passion fruit peel, habanero chili pepper waste, and carrot rejects [125,126,131]. MAE is a rapid and efficient extraction method, minimising the thermal degradation and reducing energy consumption by direct heating of the biomass, however, similarly to UAE, excessive heat can degrade the compounds of interest [125]. Industrial processing using MAE requires R&D efforts to create an economically viable protocol.

Supercritical fluid extraction (SFE) with a co-solvent has emerged as an environmentally friendly alternative. Typically, CO_2 and ethanol as a co-solvent have been used for the extraction of low-polarity compounds, for example, carotenoids [126]. Obtained extracts contain no solvent residues (if used without a co-solvent), thus providing contaminant-free extracts. By changing the operating parameters like separator type, temperature, CO_2 flow rate, the extraction cell temperature, and pressure, the selectivity of the extraction method can be modified. While a promising method, the initial costs for this high-pressure equipment can be high, and production of lower-value extracts therefore cannot be justified [126].

Deep eutectic solvents, hydrophobic or natural, are a relatively new class of green solvents formed by mixing several compounds (hydrogen bond donor and acceptor), creating a solvent with significantly lower melting point than the individual compounds used, thus forming a liquid (typically at room temperature). Natural, food-grade components are often used for lipophilic compound extraction due to their hydrophobic nature. Such eutectic solvents have been used for carotenoid extraction from orange peels and pumpkin [127,130]. Eutectic solvents are biodegradable, non-toxic and are cost-effective to create, the physicochemical properties can be easily fine-tuned to the specific needs, thus allowing for more selective extraction of the specific compounds [130]. The primary limitation of using eutectic solvents is their viscosity, which can potentially hinder the mass-transfer potential, especially in industrial-scale operations [130].

The diverse range of innovative green extraction techniques, including UAE, MAE, SFE, natural and hydrophobic eutectic solvents, demonstrates potential to enhance extraction efficiency, reduce environmental impact, and provide safe, high-quality natural pigments for various industries (Table 3). Future research should focus on optimising green extraction protocols for specific compounds and matrices, further exploring the synergistic effects of combined techniques, and investigating the toxicological and biological effects of the resulting extracts to ensure their suitability for human consumption [127–129]. Additionally, efforts are needed to bridge the gap between laboratory-scale and industrial-scale setups, particularly regarding solvent recyclability and scale-up feasibility, to fully realise the economic and environmental benefits of transforming agricultural by-products into valuable functional ingredients and natural colorants [125,127,129].

4.4. Betalains

Betalains have been found in families of the Caryophyllales, Amaranthaceae, Basellaceae, Cactaceae, Portulacaceae, Nyctaginaceae, as well as in several fungi, such as *Hygrocybe*, *Amanita muscaria*, and other sources [47,95]. Betalain concentrations in plants and microorganisms can reach up to 500 mg/100 g of biomass [50], but using genetic modification, betalain productivity can be increased [134]. From practical production potential of betalains as sources at first of significance are red beetroot (*Beta vulgaris* L.), Opuntia fruits, pitaya (*Hylocereus* spp.), and amaranth (*Amaranthus* spp.), but other species are also prospective [50]. *B. vulgaris* production volumes reach hundreds of millions of tons [135], and considering concentrations of betalains in beetroot, thousands of tons of pure betalain could be obtained [50]. Betanin isolated from *B. vulgaris* is a widely used red food colorant (Beetroot Red; EEC No. E 162), approved for use by the European Union and in the United States for use in food articles, cosmetics, and elsewhere [136]. For betalain production, food processing waste can also be used [96].

B. vulgaris and other plant betalains can be extracted by physical pressing of the red beetroots, followed by centrifugation and, to isolate pigments, methods such as thermal evaporation or osmosis can be used [137]. However, a significant part of betalains using physical processing remains in plant cells, thus to increase isolation efficiency, extraction should be applied. Betalain extraction methods briefly can be classified as conventional and non-conventional or intensive extraction methods (Table 4).

Conventional extraction methods involve the use of water or water: ethanol, acetone, or other solvent mixtures and treatments such as maceration, stirring, Soxhlet extraction, and others [138,139].

However, to increase betalain yields and prevent their degradation during treatment, extraction methods that disrupt plant cells, provide intensive mixing and, in general, intensify the extraction process are preferable (Table 4). Intensification of extraction mass mixing can be achieved using microwave or ultrasound-assisted extraction. Treatment of plant biomass in extraction solvent with waves at a wavelength from 100 μm to 1 cm (microwaves) creates internal pressure in cells and cell wall rupture, as well as intensive mixing of extraction mixtures, not requiring additional heating [142,149]. Use of microwave-assisted extraction of betalains from different biomass waste provided possibilities to obtain betalains with higher yields in comparison with conventional extraction [140].

Ultrasound generates pressure waves, creating a cavitation effect in the liquid phase, supporting solvent penetration (diffusion) into biomass and increasing the efficiency of extraction media mixing [150,151]. Micro-jets have a hydrophobic surface, and thus hydrophobic interactions during the extraction process can be increased, providing possibilities to extract low polarity substances in aqueous solvents. Ultrasound extraction can be more easily upscaled than microwave extraction, although safety concerns remain a limitation [151]. At the same time, ultrasound produces heat, which can destroy more labile compounds. Several studies have demonstrated the efficiency of ultrasound-assisted extraction [144,143].

5. Overcoming the instability of natural pigments for the food industry

5.1. Anthocyanins

The basic structure of anthocyanins is the flavylium cation, which is highly sensitive to environmental factors, leading to the degradation of the pigments and loss of colour. Glycosyl moieties, specifically the hydroxyl groups on the sugar chain, are the key targets for modification and stability improvements [152–154].

Acylation has emerged as a highly effective method for anthocyanin stabilisation – this process introduces acyl groups to the anthocyanin molecule, usually at the hydroxyl groups of the glycosyl moiety, thus forming acylated anthocyanins. There are two types of acylation – chemical and enzymatic – with enzymatic acylation being the preferred method due to its high specificity and mild reaction conditions [153,155]. Enzymatic acylation is usually done with enzymes like lipases, which catalyse the transfer of acyl groups from fatty acids or phenolic acids to the hydroxyl groups of anthocyanins [153,156]. The lipophilicity and antioxidant activity of the acylated anthocyanins have been shown to improve by the use of enzymatic acylation [157]. Often, short-chain fatty acids are used in various food-related and technological applications solutions [158]. Chemical acylation uses acylating

Table 4
Betalain extraction methods from plant biomass.

Source of betalains	Extraction conditions	Extraction solvent	Yields of betalains	Reference
Beta vulgaris	Conventional: Vortexing	Water/methanol.formic acid 84.95/15/0.05, v/v	Betacyanin 4.11 mg/g	[138]
Beta vulgaris pomace	Conventional: stirring, pH 1.5 – 5.5, T 30 – 70 °C, t 2.5 – 12.5 min	Water	Betacyanin 1.75 - 62 mg/L, betaxanthin 1.79 - 61 mg/L	[139]
Beta vulgaris peels	Microwave, pH 5.20, < 1min	Water acidified with citric acid; ethanol	Betanin 470 mg/L	[140]
Opuntia engelmannii peels	Microwave, T < 30 °C, t < 5 min	Methanol 0 – 35 %	Betalains 200 mg/g	[141]
Amaranthus tricolour leaves	Microwave, T 90 °C, t < 15 min	Water	Betacyanin 72 mg/g, betaxanthin 42 mg/g	[142]
Beta vulgaris pomace	Ultrasound, pH 5.5, T 35 °C, t 25 – 45 min	Methanol, ethanol 30 – 100 %	Increase by 30 % of betalains, betaxanthins	[143]
Hylocereus polyrhizus pulp	Ultrasound, T < 30 °C, t 20 min	Ethanol 30 %	Betalains 2 mg/g	[144]
Beta vulgaris dried peel	Maceration, 1:20 biomass:solvent ratio, 300 rpm for 1 h, 25 °C	Distilled water	Betalains 535 mg/100 g	[145]
Hylocereus polyrhizus peel	Homogenised with 95% ethanol, incubated at 40-50 °C for 60-90 min, filtered	Ethanol 95%	Betalains 2.09 mg/g peels	[146]
Opuntia ficus pulp and peel	Ultrasound-assisted extraction, 1:10 sample to solvent ratio, 10.43 min treatment time, and 31.15 °C temperature	23.15 % glycerol	Betalains 858.28 mg/L	[147]
Hylocerus polyrhizus peel	Homogenisation, centrifugation	Methanol	Betacyanin 5.591 mg/mL	[148]

agents like acyl chlorides or anhydrides in the presence of a catalyst, compared to enzymatic acylation, chemical acylation is done in harsher conditions [153,159]. Acylation enhances the stability of the anthocyanins by reducing the nucleophilic attack on the flavylium cation against light, temperature, and pH changes, and the improved solubility in lipid-based matrices creates the possibility to use them in food and cosmetic products [155,160]. In addition to acylation, several other chemical modification strategies have been proposed. Methylation of anthocyanins involves the modification of hydroxyl groups of the anthocyanin molecules with methyl groups – this type of modification improves the stability in low pH [62,160].

Several technological approaches to stabilise anthocyanins exist. Most commonly, anthocyanin-rich extracts are encapsulated in a protective matrix to shield them from the environmental effects (pH, temperature, moisture, oxygen) (Fig. 8). The encapsulating matrices used are protein isolates, maltodextrins, and other polysaccharides, in combination with drying methods, such as freeze- or spray-drying. Micro- and nano-encapsulation improves the shelf-life and colour intensity of the pigments [161].

Copigmentation enhances the stability and colour intensity of anthocyanins. Copigmentation of anthocyanins can be divided into two types: intramolecular and intermolecular. Intramolecular copigmentation happens when the same anthocyanin self-associates, while intermolecular copigmentation occurs when the anthocyanin molecule interacts with phenolic acids or metal ions (creating metal-anthocyanin complexes) [162,163]. Excellent stability improvements have been achieved by combining copigmentation and encapsulation, allowing for an increase of the colour stability and retention [164].

Stabilisation efforts of anthocyanins mostly concentrate on the use of these pigments in the food industry as natural colourants or as part of functional foods [160,165]. Since anthocyanins also exhibit strong antioxidant activity, they have been used as bioactive components in pharmaceuticals [166]. Stabilised anthocyanins have different solubility than their native forms, thus the use of these molecules is also extended in cosmetic products, which are more lipophilic in nature [167]. Anthocyanin complexes have also been extensively studied in the context of their use in intelligent packaging – the visual change of colour depending on the pH can be used to indicate the freshness of produce, meat, or other products [168,169]. Despite the general acceptance and progress made towards stabilisation of anthocyanins, several challenging aspects remain (Fig. 8). Stability advantages offered by acylation are met with the issues in scalability and the overall costs of the final product, making this approach not viable. In addition, the use of modified anthocyanins requires the approval of regulatory bodies, firstly to ensure safety and secondly to eliminate any possible toxicity issues, which is a time-consuming and costly process. Nevertheless, work should continue on these aspects, since technological advancements will allow to optimise stabilisation reactions and materials to improve the yields and produce higher quality end-products.

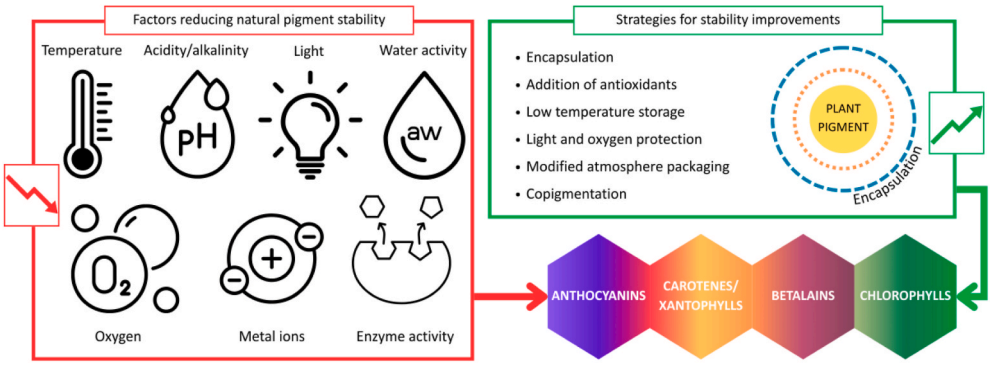

Fig. 8. Factors influencing natural pigment stability and strategies for stability improvements.

5.2. Chlorophylls

Chlorophyll's inherent instability under environmental factors such as light, heat, pH, and exposure to oxygen can limit their broader application in food and food systems [170]. Several stabilisation strategies that can effectively preserve the colour of chlorophylls have been described, however, the lack of standardised evaluation protocols does not allow for full evaluation of the possible applications of the stabilised chlorophyll (Fig. 8).

Encapsulation is the most promising and most researched approach for the stabilisation of chlorophylls – up to 90-97% encapsulation efficiency has been achieved [171]. Encapsulation involves surrounding the chlorophyll molecule with a protective material, for example, proteins, carbohydrates, or lipids, thus creating "capsules" that shield the pigment from extrinsic factors. Encapsulation methods such as spray drying with inulin-whey protein or freeze-drying with maltodextrin and whey protein isolate have shown high efficiency of encapsulation. Carrier materials such as whey protein, maltodextrin, and polysaccharides (e.g., sodium alginate, carrageenan) influence encapsulation efficiency and thermal stability of chlorophyll microcapsules [171,172]. Although spray drying, as an encapsulation method, holds more promise for industrial applications due to the possible scaling of production, freeze drying shows superior encapsulation efficiency, particularly for applications where pH and light stability are important [173]. Encapsulation increases thermal and photostability with practical application of the created ingredients in dairy products [173,174]. Encapsulation of chlorophylls in nanoemulsions can increase the hygroscopicity, bioavailability, and stability even further [175,176], expanding the concepts of pigment stabilisation beyond conventional encapsulation [175]. Encapsulation efficiency and experiments, where the stability has been demonstrated, proves that the microenvironment modification is critical for pigment stability [172]. Spray-drying microencapsulation of chlorophyll using whey protein isolate (WPI) markedly improves chlorophyll stability, water solubility, and antioxidant activity by protecting the pigment from thermal, oxidative, and environmental degradation [177,178]. Spectroscopic analyses (FT-IR, fluorescence, XRD) indicate strong interactions between chlorophyll and WPI—primarily via hydrophobic interactions and hydrogen bonding - leading to reduced crystallinity and enhanced structural stabilisation of chlorophyll within the protein matrix [178]. Increasing WPI ratio and optimising inlet air drying temperature result in higher encapsulation efficiency (>95%), lower moisture content, and improved retention of antioxidant activity during storage [177,178]. Overall, WPI-based microencapsulation is an effective strategy to enhance the physicochemical stability and functional performance of chlorophyll for food and bioactive applications.

Metal ion replacement, particularly with Zn^{2+} and Cu^{2+}, has emerged as a highly effective stabilisation strategy that enhances both thermal and acid stability of chlorophyll [62,179]. This approach involves substituting the central magnesium ion in chlorophyll with more stable metal ions, creating chlorophyll derivatives with improved resistance to environmental stressors [180]. The combination of metal ion substitution with high hydrostatic pressure processing creates synergistic effects that significantly improve colour stability and antioxidant activity [181,182]. This dual approach addresses multiple degradation pathways simultaneously, providing protection for chlorophyll in food applications by stabilising the chlorophyll structure against different stressors and improving the thermal stability [182]. Copper chlorophyllin is commercially available and approved by the food safety authorities in the EU, Australia, the UK, and several other regions, however in the US, the use of copper complexes of chlorophyll are prohibited for use in food. While copper chlorophyllin is widely used in different products, due to the changes it has to undergo to increase its stability (the exchange of Mg to Cu), it is not considered as a natural pigment, thus it is still important to research new possibilities of chlorophyll stabilisation with other additives and methods.

Hydrocolloids such as pectins, xanthan gum, gum Arabic, or carboxymethyl cellulose have been used to stabilise chlorophyllin in acidic environments by the formation of electrostatic and hydrogen bonds, which reduces the particle size and improves dispersion [182,183]. Hydrocolloid stabilisation improves the colour retention without changing the pH [182]. Emulsifiers like sucrose fatty acid ester or saponins promote chlorophyll aggregation thus protecting it against photodegradation in aqueous systems [184]. By combining nanostructured carriers with hydrocolloids or emulsifiers, it is possible to achieve synergistic chlorophyll stabilisation effects suitable for industrial applications [185]. While a promising technique, the interaction mechanisms between the hydrocolloids/emulsifiers and chlorophyll have not been fully investigated and require further optimisation. Some of the stabilising agents may affect the flavour and texture and could potentially give radically different colouring and stability properties depending on the food matrix. Also, the health implication of some of the investigated emulsifiers can limit their application [183,184].

The importance of emerging green extraction technologies must also be mentioned in the context of chlorophyll stabilisation, as the extraction of chlorophyll from food or agro-waste biomass is the stage the most susceptible to degradation [186]. Non-thermal extraction techniques, such as enzyme-assisted extraction and supercritical CO_2 extraction, allow to obtain solvent-free pigments with additional antioxidants, at the same time aligning with the sustainability goals and supporting circular economy initiatives [187]. Extraction using the mentioned methods helps in preserving chlorophyll due to the required low temperatures for the process. Enzyme-assisted extraction can be combined with metal ions (e.g., Zn) to enhance the chlorophyll yield and colour quality [188]. Although promising, these environmentally friendly extraction methods, which contribute to higher pigment stability, lack industrially justified experiments, additionally, the cost and activity consistency should be evaluated [189].

Despite advances in chlorophyll stabilisation several limitations still exist that hinder industrial adaptation. The primary concern is the scalability: while several techniques demonstrated on lab-scale show promising results, the upscaling to commercial-scale faces challenges due to the costs of the operation, materials, or complexity of the suggested approaches. Long-term stability across different food matrices complicates the creation of one-fits-all solutions due to the different environmental factors which lower the stabilisation effectiveness. However, integration of multiple techniques shows potential synergistic stabilisation effects, but it comes with increased complexity and costs of the process. Adaptation of novel stabilisation techniques requires techno-economic analysis and evaluation of commercial viability to provide a comprehensive stabilisation framework of chlorophyll for the food industry.

5.3. Carotenoids

Carotenoids are widely used in the food industry due to their colouring properties and health benefits, however, challenges relating to their chemical instability, poor solubility in water, and susceptibility to oxidation require methods for stabilisation that would mitigate these effects (Fig. 8). The conjugated double bonds within the carotenoid molecules provide them the bright hues, however, they make these molecules chemically unstable and prone to oxidation [190,191]. Encapsulation and nanoencapsulation techniques are used to protect the double bonds by creating a physical barrier against oxygen, light, and heat [192,193]. Supramolecular complexes created with materials like alginate, chitosan, or gelatine can modulate the release kinetics, improve the bioavailability of carotenoids, and alsosupport their stability [193]. Incorporation of antioxidants into carotenoid-containing formulations has also been shown to improve the shelf life of products in relation to carotenoid contents [191]. While these strategies enhance the stability and bioavailability of carotenoids, challenges remain in terms of scalability and regulatory approval for commercial use.

Micro- and nano-encapsulation in combination with spray drying are

the primary methods studied and investigated for stabilisation of carotenoids to prevent their degradation in light, heat, oxygen, and various processing conditions. Studies indicate that the choice and combination of different wall materials used can impact the stability of carotenoids, encapsulation efficiency, and their controlled release, and thus bioavailability [194]. Various wall materials have been used, for example, maltodextrin, gum Arabic, modified starches, proteins, polysaccharides, and carbohydrates [195]. Optimisation of encapsulation parameters, such as the wall material concentration, drying temperature, and ratios of wall materials when used in mixtures, is crucial for enhancing thermal stability and preservation of colour during storage [196–198]. It has been shown that using microencapsulation can improve the β-carotene retention by up to 70-90% [194–196]. Nanoencapsulated lycopene has been shown to retain 80% of its concentration under accelerated storage conditions, and at the same time, improved bioavailability was observed [199], nanoencapsulation can ensure 80-90% pigment retention when polymeric nanoparticles or cyclodextrins are used [190,200,201]. Nanoencapsulated carotenoids show superior stabilisation effect as compared to microencapsulation due to the smaller particle size and larger surface area [202]. Despite shown retention and promise in carotenoid stabilisation, the variability in encapsulation efficiency across studies shows differences in wall materials, carotenoid sources, and overall drying parameters, influencing the final result. The influence of encapsulation on sensory attributes is largely underexplored, limiting the practical applications.

Pickering emulsions stabilised by polysaccharides or proteins have been developed for carotenoid encapsulation, which improve the physical and chemical stability, offering enhanced bioaccessibility and controlled release [203,204]. Emulsifiers and surfactants, like zein or Tween, have been shown in various emulsion systems to enhance lutein and β-carotene stability, improve sensory attributes, and protect from various degrading factors [205,206].

Combined encapsulation techniques show promise in enhancing the carotenoid stability even further. Different mixtures of wall materials can be used, requiring optimisation of the wall material ratios [205]. Addition of antioxidants to the encapsulation matrix has been demonstrated to improve the stability of encapsulated carotenoids. Incorporation of antioxidants, for example, ascorbic acid, phenolic acids, and tocopherols, has been demonstrated to synergistically improve the carotenoid stability by scavenging free radicals and reducing oxidation [207,208]. Using antioxidants as additives in encapsulation can reduce the degradation under UV irradiation, thus improving the shelf life of products [203]. The complex interactions between wall material-pigment-antioxidant are not fully investigated, thus complicating the optimisation of formulations [208]. Also, since antioxidants typically have a distinct taste, the sensory properties should be evaluated in combination with the regulatory aspect prior to incorporation in food matrices [197].

Carotenoids encapsulated in different matrices have been used in various food products, such as cookies, yoghurt, beverages, confectionery, and even 3D printed foods [205,209,210]. The main positive demonstrated effect is the preservation of colour and antioxidant activity, giving the product an additional functional property [204,206]. Despite efforts to transfer the lab-scale development to pilot or industrial scale production, several aspects still remain unexplored – sensory attributes, texture when the stabilised material has been added to the final food matrix, long-term stability, and consumer acceptance [197,211].

5.4. Betalains

The factors influencing betalain stability are pH of the environment [212], presence of antioxidants [213], use of encapsulation agents [214], chelators [215], and elevated temperatures [216]. Stabilisation of betalain molecules happens through the neutralisation of the electrophilic centre of the molecule by the interaction with the charged nitrogen (antioxidants, chelators) or by creating inclusion complexes acting thus protecting the pigment (dextrins) [97].

Betalain stability is usually improved through encapsulation (Fig. 8). Different encapsulating agents have been used, like cellulose-based materials [217], polysaccharides, proteins [50]. Most frequently used polysaccharides for the encapsulation of betalains are maltodextrin [218], guar gum, gum Arabic [219], xanthan gum, pectin [146].

The stability of betalains can be improved through copigmentation. A recent study on an underutilised, betalain-containing *Celosia cristata* L. flowers indicated that betalain stability can be increased through copigmentation with gum Arabic, pectin, whey protein ascorbic acid, and calcium carbonate in the concentrations 0.33, 0.33, 0.33, 0.05, and 0.01%, respectively [220]. Betalain stability and thermal stability were significantly increased during the storage period tested [220]. Betalain stability is known to be pH dependent – at alkaline conditions, pH above 10, betalains tend to degrade and a bathochromic shift occurs [221]. Maltodextrin as an encapsulating agent was demonstrated to increase the pH stability of betalains [218].

Microcrystalline cellulose has been proposed as a natural encapsulating agent, which provides advantages over the more commonly used polysaccharide materials – the degree of crystallinity and degree of polymerisation, particle size, shape, thermal stability, and porosity, as well as the large surface area and moisture retaining characteristics. Betalain encapsulation using microcrystalline cellulose as the wall material was shown to promote storage stability of betalains at 4 and 27 °C in light and dark conditions, thermal stability was improved at 80 and 100 °C at various pH (1.2 – 7.4 pH) and water activities (0.089 and 0.898), indicating the potential of this encapsulating material in natural pigment encapsulation [217].

In recent years, pigment stabilisation has emerged as a distinct and rapidly expanding research domain, with microencapsulation and nanoemulsion-based systems receiving particular attention [222,223]. Microencapsulation using biopolymeric carriers, including polysaccharides, proteins, lipids, and their combinations, has been widely explored to improve the resistance of natural pigments against light, oxygen, temperature fluctuations, and pH variation, while enhancing handling properties and compatibility with diverse matrices [96,178,224]. Similarly, nanoemulsions and other nanostructured delivery systems have been developed, particularly for lipophilic pigments such as carotenoids and chlorophylls, enabling improved dispersibility, colour uniformity, and oxidative stability in aqueous formulations [222,225]. Collectively, these approaches demonstrate clear potential to mitigate intrinsic pigment instability and broaden application possibilities across food, cosmetic, and pharmaceutical systems. Most stabilisation studies are conducted at laboratory scale and rely on short-term storage experiments, providing limited insight into long-term stability, degradation mechanisms, and performance under real processing and formulation conditions [96,223]. Furthermore, comparative assessments across stabilisation strategies are scarce, and standardised protocols for evaluating stabilisation efficiency, release behaviour, and matrix interactions are largely lacking [96,224]. Scale-up feasibility, cost-efficiency, and regulatory considerations, particularly, for nano-enabled systems, remain insufficiently addressed, limiting industrial uptake despite promising laboratory results [223,225]. Addressing these limitations through application-oriented, comparative, and pilot-scale studies is essential for translating stabilisation advances into industrially viable solutions and informing about future perspectives in the field.

6. Conclusions and future perspectives

Research on natural pigments and their extraction from food, agro-food, and biowastes has become an important field of study, driven by the potential to address the environmental aspects of waste valorisation, waste reduction, and satisfying ever-increasing consumer demand for natural, health-promoting additives. These techniques not only present higher extraction yields, but also better preservation of the bioactivities

related to the retrieved pigments and reduced energy consumption, as well as solvent use, thus aligning with green chemistry and circularity principles. Biomass sources potentially used for pigment extraction, such as agro-industrial wastes and food wastes, reflect the vast untapped potential of, at least a part of, nearly 1.64 billion tonnes of annual global food waste. Additionally, recent research focuses on biosynthetic and microbial fermentation methods to produce genetically enhanced biomass for pigment extraction – biotechnologies offer scalable, season-independent alternatives for pigment extraction, although these methods are still constrained by the high production costs, safety concerns, and complex regulatory landscapes. Despite the recognised environmental and functional advantages of natural pigments retrieved using green extraction technologies, challenges in pigment stability, the economic feasibility, and scalability of the extraction systems remain to be demonstrated. The emphasis of the research in the natural pigment field depends on the disciplinary perspectives and maturity levels of the technologies used, which is connected to the type of biomass utilised, whether it is microbial, algal, biotechnologically produced, or plant-derived. Commercialisation of natural pigments is further complicated due to the regulatory considerations and consumer acceptance, with many studies highlighting the need for more comprehensive frameworks to ensure safety and efficiency. The trade-offs between the yield, bioactivity, and sustainability of the proposed processes underscore the lack of integrated assessment protocols that can fully consider environmental and economic aspects (Fig. 9). In particular, pigment stabilisation remains one of the most critical unresolved bottlenecks limiting the translation of laboratory-scale advances into commercially viable products. Natural pigments are inherently sensitive to light, oxygen, temperature, pH, and matrix composition, and current stabilisation strategies often fail to provide sufficient protection under real processing and storage conditions [2,222]. While encapsulation, nanoemulsions, copigmentation, and biopolymer-based carriers have demonstrated improved stability at laboratory scale, there is limited mechanistic understanding of pigment–carrier interactions, diffusion behaviour, and degradation pathways within complex food and cosmetic matrices [96,224]. Overall, while natural pigment research offers strong opportunities, significant knowledge gaps must still be addressed to enable economically and ecologically viable biomass valorisation into stable, application-ready pigments.

Recent studies show advancements in green extraction techniques, such as ultrasound-assisted, microwave-assisted, supercritical fluid extraction, and the use of natural deep eutectic solvents, which offer higher pigment yields, reduced solvent use, and enhanced bioactivity preservation, aligning with circular economy and green chemistry principles [226,180,227,228]. Complementary approaches, such as pulsed electric field, cold plasma, hydrostatic pressure, and nanotechnologies, can further improve stability, extraction selectivity, and functionality of the pigments [225,229]. Environmentally, these methods reduce landfill burden, mitigate greenhouse gas emissions, and lower ecological footprints by minimising toxic solvent use and energy consumption [230,231]. Despite the advancements and adaptation of different techniques in natural pigment research, challenges persist in industrial scalability, high initial investment needs, and the requirement for specialised equipment, particularly when optimising processes for

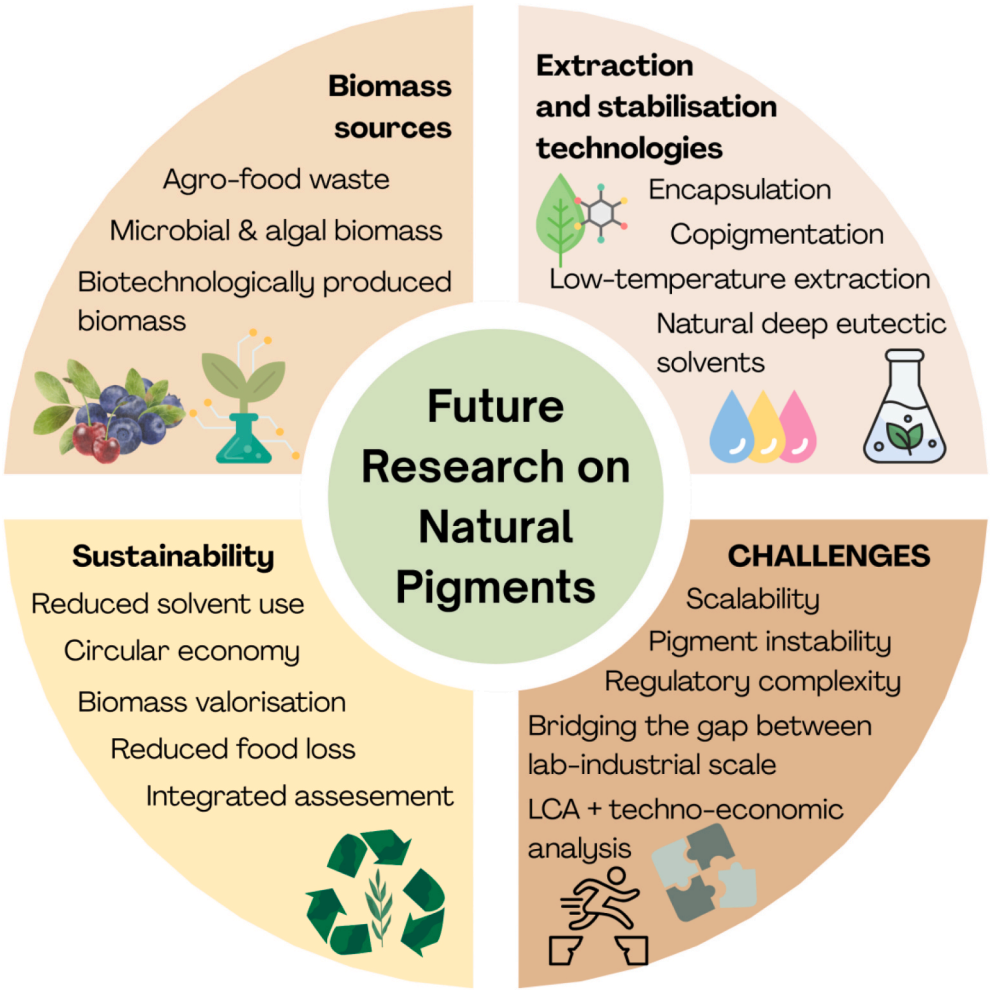

Fig. 9. Main aspects and future perspectives of natural pigment research.

diverse sources of biomass [232]. Additionally, natural pigment stability during and post-extraction remains a limiting factor for application [2]. Most encapsulation and stabilisation studies remain confined to short-term storage tests and small batch sizes, with limited pilot-scale validation and scarce data on long-term stability, batch-to-batch variability, and cost-efficiency under industrially relevant conditions [96,223]. Future research should therefore prioritise systematic, comparative evaluations of encapsulation materials and stabilisation strategies using identical pigments across multiple matrices, alongside pilot-scale trials that integrate extraction, stabilisation, and formulation steps. Biotechnological methods for obtaining biomass for pigments offer a promising alternative for scalable pigment production, for example, for astaxanthin, however, there are disagreements over which methods are the most practical for application and industrial scaling, therefore, more realistic are the approaches where plant-derived biomass is used [233,234]. Bridging the gap between lab-scale experimental setups and industrial implementation requires thorough pilot-scale studies to evaluate the commercial potential of sustainable pigment production [231]. The absence of thorough techno-economic and life cycle assessments limits understanding of long-term sustainability and market feasibility of natural pigment production [2,223].

Regulatory frameworks within the natural pigment sector are evolving gradually in order to address the safety, toxicity, labelling, and quality standards, and take into consideration the development of new extraction methods and biotechnological solutions for pigment production [5,227,229]. While deep eutectic solvents align with the safety and sustainability goals, evaluation of their toxicity remains a challenge, particularly for novel solvent systems with unknown synergistic evaluations. Although natural pigments are generally perceived as safe alternatives to synthetic dyes and offer health benefits that support their inclusion in functional foods [235,236], regulatory approval is often delayed due to fragmented global standards and incomplete risk assessments. The lack of harmonised regulatory guidance also affects the adoption of novel encapsulation and nanostructuring approaches, as uncertainties regarding material safety, labelling, and nano-specific risk assessment can discourage industrial uptake and investment [223,225]. The limitation of novel product regulatory acceptance can limit the R&D efforts in certain regions, meaning that innovations will flow to the regions with more liberal regulatory burdens, and thus, the inventors, scientists, and entrepreneurs will follow their inventions. Regulatory acceptance is crucial for innovation uptake and a lack of organised frameworks can lead to loss of progress within the field and further re-importing of the innovations to where they originated. Consumer acceptance and compliance with food safety regulations are essential for market success, yet discussions on harmonised international guidelines, standardised labelling, and safety testing protocols are often insufficiently detailed. As regulatory clarity is crucial for market access, international collaboration among scientists, industry, and policymakers is needed to establish unified frameworks that support commercialisation while ensuring public health protection.

Stability of natural pigments remains a critical challenge limiting the practical application and commercialisation of natural pigments, due to their sensitivity to various abiotic factors [2,226]. Innovative stabilisation techniques such as encapsulation, nanostructuring, and copigmentation have shown potential in enhancing resistance to degradation, improving shelf life, functional aspects, and overall appeal in various matrices [222,224,225]. Extraction techniques that avoid the use of elevated temperatures contribute even further to preservation of pigment colour integrity [180]. In the context of large-scale pigment production from food processing by-products and agro-industrial residues, contamination risks associated with raw material heterogeneity, pesticide residues, heavy metals, and microbial load must be carefully considered. Future research should therefore integrate contamination monitoring, pre-treatment strategies, and compliance with food and feed safety regulations into extraction and stabilisation workflows, alongside techno-economic and life cycle assessments, to ensure safe and regulatory-compliant industrial implementation. Similarly to the extraction, also in stabilisation, the scalability remains a challenge – the stabilisation approaches remain insufficiently validated for real-world industrial applications, only a few pilot-scale studies have explored these aspects [96]. While the research supports the potential of advanced stabilisation techniques to overcome degradation of natural pigments, the method of choice can differ based on the application. Comparative studies and standardised protocols are needed to evaluate the stabilisation efficiency in different matrices.

Despite extensive investigation of encapsulation and stabilisation strategies, several unresolved technical challenges remain insufficiently addressed. Many carrier systems provide limited protection against oxidative and photo-induced degradation, particularly under thermally or mechanically demanding processing conditions. In addition, pigment–carrier interactions, release kinetics, and degradation mechanisms within complex matrices remain poorly understood, limiting the rational design of stabilisation systems tailored to specific pigment classes. Future research should therefore prioritise comparative, application-oriented studies that evaluate stabilisation efficiency, degradation pathways, and release behaviour across different matrices, coupled with pilot-scale validation and techno-economic assessment. However, the scalability of these stabilisation techniques remains insufficiently demonstrated, as only a limited number of studies have evaluated their performance beyond laboratory scale or under real industrial processing conditions. Addressing these gaps will require the development of standardised testing protocols, integration of techno-economic and life cycle assessments, and closer collaboration between academia and industry.

CRediT authorship contribution statement

Linards Klavins: Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Alise Zommere:** Visualization, Formal analysis. **Taisija Gricenko:** Formal analysis. **Maris Klavins:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The authors are unable or have chosen not to specify which data has been used.

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