Sustainable green extraction of anthocyanins and carotenoids using natural deep eutectic solvents (NaDES): A review of recent developments

Jennifer Osamede Airouyuwa1[1,†], Nilushni Sivapragasam[1 †], Ali Ali Redha[2, 3], Sajid Maqsood[1,4*]

[1] Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain 15551, United Arab Emirates

[2] The Department of Public Health and Sport Sciences, University of Exeter Medical School, Faculty of Health and Life Sciences, University of Exeter, Exeter, UK

[3] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, QLD, Australia

[4] Energy and Water Center, United Arab Emirates University, Al-Ain 15551, United Arab Emirates

*Corresponding author: Sajid Maqsood (sajid.m@uaeu.ac.ae)

[†]Authors with equal contribution

Abstract

Recently, deep eutectic solvents (DES) have been extensively researched as a more biocompatible and efficient alternative to conventional solvents for extracting pigments from natural sources. The extraction efficiency of DES extraction for the anthocyanin and carotenoid extraction can be enhanced by microwave-assisted extraction (MAE) and/or ultrasound-assisted extraction (UAE) techniques. Apart from the extraction efficiency, the toxicity recovery of the pigments and the bioavailability are crucial for potential applications. A plethora of studies are presently examining the extraction efficiency, toxicity, and recovery of pigments from various natural plant-based matrices using DES. Nevertheless, a detailed review of the deep eutectic solvent extraction of natural pigments has not been reported to date. Additionally, the toxicity, safety, and bioavailability of the extracted pigments, and their future potentials are not thoroughly documented. Therefore, this review is designed to understand the aforementioned concepts in using DES for anthocyanin and carotenoid extraction.

Keywords: anthocyanins; carotenoids; deep eutectic solvents; natural deep eutectic solvent; pigment extraction; green extraction.

1. Introduction

The history of pigments dates back to ancient Egyptians who used *Rubia cordifolia* for dying the textile (Orna & Fontani, 2022). Since then the use of pigments has been explored in the food industry, biomedical sciences, and cosmetic industry, and is well-studied for the prevention and treatment of diseases. The exponential growth in these industries, over the years, has demanded the production of pigments. According to Future Market Insights, the market value for the production of synthetic pigments was USD 5,862.0 million and is anticipated to have a growth rate of 4.5% by the year 2032, which accounts for USD 9,824.9 million (Future Market Insights, 2021). However, the production of synthetic pigments is expected to grow by 76% while natural pigment production is predicted to have a 24% fall (Rodríguez-Mena et al., 2022). The major factor that affects the decline in natural pigment production is the instability of the natural pigments to light, heat, oxygen, organic compounds, metal ions, and pH (Rodrigues et al., 2020; Shen et al., 2022). Moreover, unlike synthetic pigments, significant amounts of natural pigments are required for the production of equal color strength. Nevertheless, due to the obvious toxic effects associated with synthetic pigments upon prolonged usage, natural pigments are gaining attraction in recent years to partially or completely replace synthetic pigments (Di Salvo et al., 2023). As a result, many studies are being explored to investigate the efficient extraction process and the chemical intactness of the natural pigments.

The conventional extraction methods used in the extraction of natural pigments are simple, yet provide poor extraction efficiency. Furthermore, the type of extraction solvents and the temperature of extraction are two vital parameters that vastly affect extraction efficiency. The increased amounts of extraction solvents required for efficient pigment extraction can lead to toxicity, flammability, and non-biodegradability (Jacobsen et al., 2019). To overcome the

challenges in conventional extraction methods, innovative extraction techniques such as microwave-assisted extraction, ultrasound-assisted extraction, pulse electric field, and superficial extraction techniques are investigated (Rodríguez-Mena et al., 2022). Similarly, the use of neoteric extraction solvents such as DES and its subclass NaDES are explored as a substitute for conventional extraction solvents (Liu et al., 2019).

Considering the increased need for natural pigment and the efficient extraction process, this review aims to comprehend the different extraction methods used for the recovery of anthocyanins and carotenoids– with a major focus on DES/NaDES as green extraction solvents **(Figure 1)**. Besides, the toxicology and safety of the anthocyanins and carotenoids extracted using DES/NaDES were discussed followed by their usage in the food industry and the future perspectives and limitations.

2. Conventional and novel extraction methods of natural pigments

The extraction of natural pigments from plants using conventional methods such as Soxhlet, maceration, percolation, and solvent extraction methods have been widely studied (Lotfi et al., 2015). These conventional methods are time-consuming, require large amounts of extraction solvents, non-cost effective, and are mostly non-specific (Jacobsen et al., 2019). The choice of organic solvents used in the extraction of pigments is dependent on the class of pigment. On one hand, polar pigments like anthocyanins are extracted with ethanol, methanol, and water (Monrad et al., 2010). On the other hand, non-polar pigments like β-carotene and lycopene, which are highly lipophilic carotenoids require non-polar solvents like hexane and acetone. β-carotene and lycopene lack polar functional groups due to the conjugated hydrocarbon structures (Saini & Keum, 2018). These conventional solvents are toxic, highly volatile, flammable, and can negatively impact the

environment (Zainal-Abidin et al., 2017). Similarly, there have been reports of poor stability of the pigment and extract obtained by conventional solvents (Martins et al., 2016). Hence, there is a pivotal need to develop a suitable solvent that is stable, safe, and efficient (Z. Yu et al., 2022a). Currently, significant progress has been made to overcome the challenges in extracting pigments.

Ionic liquids are one the most explored solvents for the extraction of pigments. ILs are synthesized by various anions and organic cations. They have found useful applications in the extraction of pigment, bioactive compounds, biomolecular research, pharmaceuticals, and organic synthesis (Cao et al., 2018). A study by Murador et al. (2019) evaluated the extraction yield of carotenoid from orange peel using ILs compared to conventional solvent. The carotenoids obtained from ILs were more stable with higher antioxidant activity compared to conventional solvents like acetone. However, ILs are highly priced, and hence utilizing ILs for the extraction of pigment is not a feasible solution, especially at a larger scale (Abushammala & Mao, 2020). In addition, some studies reported a high-level of toxicity and impaired biodegradability of ILs (Salam et al., 2016). Due to the constraints associated with organic solvents and ILs, new alternative solvents are being explored. These solvents are chosen based on their health and environmental toxicity, biodegradability, cost, and extraction efficiency.

Deep eutectic solvent (DES), which was first synthesized by Abbott et al. (2004) is a new class of solvent that is promising for pigment extraction. DESs are analogues to synthetic ILs and are mostly obtained from various natural sources, which makes them suitable for the green extraction of pigments. Mainly, plant metabolites and/or cellular constituents are extracted and mixed in different proportions to obtain eutectic mixtures. Interestingly, each constituent of the eutectic mixture has a higher melting point, however, when they are mixed together, the components will

melt at a much lower temperature (Dai et al., 2013). For example, in a DES that consists of choline chloride and urea, the melting points of choline chloride and urea are 302 and 133 °C, respectively. When these two components are mixed, the mixture melts at less than 60 °C while the freezing point depression occurs at 12 °C this temperature is known as the "eutectic temperature" and the mixture is known as the "eutectic mixture". Since choline chloride and urea are obtained from natural sources, these solvents are labelled as "natural deep eutectic solvent (NaDES)" (Ijardar et al., 2022; Ling & Hadinoto, 2022). The possibility of these solvent mixtures melting at a much lower temperature, than their melting points, is attributable to the intermolecular interaction between the HBD and the HBA which expands the size of the NaDES complex and reduces the electrostatic interactions consequently the energy required to melt the NaDES is reduced (Pan et al., 2021). Depending on the nature of constituents, the DES can be classified into five types: Type I (mixture of quaternary ammonium salt and metal chloride); Type II (mixture of quaternary ammonium salt and hydrate of metal chloride); Type III (mixture of quaternary ammonium salt as hydrogen bond acceptor (HBA) and amines, amides, carboxylic acids, alcohols, and sugars as hydrogen bond donor (HBD) to form ionic NaDES); Type IV (mixture of metal chloride and any HBD); and Type V (mixture of non-ionic HBD and HBA) (Choi et al., 2011). Nevertheless, type III has been widely explored in recent years for its selectivity in extracting various components and has been substantiated with less toxicity. In a type III eutectic system that consists of choline chloride and urea, the choline chloride acts as HBA and urea acts as HBD. Through the intermolecular hydrogen bond interactions, the lattice energy of the mixture decreases– requiring a lower temperature to melt, resulting in a lower eutectic temperature. Figure 2 illustrates the interaction in a eutectic mixture and the potential to selectively extract the pigments.

3. Extraction of anthocyanins using DES/NaDES: influential factors

Anthocyanins are a class of polyphenols mainly found in fruits, vegetables, and flowers. Due to the substantial health benefits manifested by anthocyanins, these compounds are incorporated into foods to be used as functional foods. Besides, anthocyanins are added to foods as natural colorants replacing synthetic food colorants (Dai et al., 2016). In light of such facts, extracting anthocyanin, from natural sources, using type III DES/NaDES has gained a lot of attention due to the intermolecular interactions that can be formed between the hydroxyl and carbonyl groups of anthocyanins with the eutectic mixture (Alrugaibah et al., 2021). The intermolecular interactions are highly influenced by pH, polarity, viscosity, water as the co-solvent, and the solid-to-solvent of the eutectic system. Furthermore, to enhance the extraction efficiency of anthocyanins, integrative techniques such as microwave-assisted extraction (MAE) (Evitasari et al., 2022; Han et al., 2023) and ultrasound-assisted extraction (UAE) (Jovanović et al., 2022; MacLean et al., 2021; Thakur et al., 2022; Velásquez et al., 2021) are commonly used along with the DES. The temperature and the duration of the treatment of these techniques greatly influence the degree of anthocyanin extraction. Collectively, the nature of type III DES/NaDES and the mechanical extraction techniques used significantly affect the extraction efficiency of anthocyanins. The structure of some anthocyanins is depicted in **Figure 3** showing the variation in the functional groups in their structure. The proceeding section will illustrate the effects of individual parameters on the extraction efficiency of anthocyanins.

3.1 Effect of pH on DES/NaDES extraction of anthocyanins

Depending on the pH, anthocyanins can exist in various forms. At acidic pH <4, pH 6-8, and pH >8, anthocyanins exist as cation flavylium, quinoidal base, and chalcone, respectively (Bosiljkov et al., 2017). Since these species differ in their functional groups, the interaction between the anthocyanins and the DES will highly depend on the pH of the DES. The DES/NaDES that contain organic acids have the potential to stabilise the flavynium ion because these acids can stabilize the phenolic groups in C4', C5, and C7 (Dangles et al., 2018). In contrast, when alkaline groups such as amide or amine are present as HBD in the DES/NaDES, the flavynium cation can be destabilized which in turn reduces the extraction efficiency (Khoo et al., 2017; Bosiljkov et al., 2017). When bilberry anthocyanin was extracted using choline chloride-lactic acid and choline chloride-urea, the total anthocyanin content was 2.5 and 0.9 mg CGE/ g DW respectively (Jovanović et al., 2022). The alkaline pH of choline chloride-urea causes the destabilization of anthocyanin resulting in poor anthocyanin extraction. In terms of anthocyanins from Chilean berries (*Luma chequeen*), the total anthocyanins were high in lactic acid-glucose DES (3.3 mg/g DW) compared to tartaric acid-glycerol (0.8 mg/g DW) due to the comparative acidic pH of lactic acid-glucose (Velásquez et al., 2021). Similarly, when the anthocyanin was extracted from black rice bran powder using various DES, the highest and the lowest anthocyanin were obtained with lactic acid-fructose (32.5 mg/L) and sucrose-fructose-glucose (7.5 mg/L), respectively (Thakur et al., 2022). Other studies have also reported high yield of anthocyanins extracted from several plant sources using acid-based DES such as perilla leaves (Han et al., 2023), wine lees (Bosiljkov et al., 2017), blackberry (Zannou & Koca, 2022), grape skin (Iannone et al., 2021), blueberry (da Silva et al., 2020), and mulberry (Guo et al., 2019). Thus, the anthocyanin extraction can be enhanced by using an acid-based DES.

3.2 Effect of DES/NaDES polarity on the extraction of anthocyanins

Anthocyanins are polar compounds due to the rich hydroxyl and carbonyl groups. Hence, they dissolve well in polar DES/NaDES due to polar-polar interactions. The raspberry anthocyanins were extracted more in choline chloride-1,4-butanediol compared to choline chloride-glycerol, choline chloride-glycol, and choline chloride-glucose, due to the high polarity of choline chloride-1,4-butanediol (Xue et al., 2020). The extraction of black rice bran was favored in a lactic acid-fructose eutectic mixture compared to a mixture that had sucrose-fructose-glucose (Thakur et al., 2022). Various other studies with blackberry (Zannou & Koca, 2022), blueberry (da Silva et al., 2020), and chokeberry (Jovanović et al., 2023) have also shown high extraction efficiency of anthocyanins in highly polar DES. The increased polar-polar interactions are owed to the hydrogen bonds that forms between anthocyanins and DES/NaDES– to enhance the extraction.

3.3 Effect of DES/NaDES viscosity and water content on the extraction of anthocyanins

The viscosity of the DES/NaDES plays an important role in the extraction and stabilization of the anthocyanins. In this section, only the effect of viscosity on the extraction of anthocyanin will be discussed and the stability will be discussed in the later section. When the viscosity of the DES/NaDES is high, the molecular movement gets hampered making the solid-solvent interaction less, thus resulting in less extraction efficiency (Han et al., 2023; Zannou & Koca, 2022). For example, the bilberry extract showed high anthocyanin extraction in a choline chloride-lactic acid mixture compared to choline chloride-citric acid, choline chloride-malic acid, and choline chloride-tartaric acid (Jovanović et al., 2022) although lactic acid is a monoprotic acid. This

observation could be attributed to the higher viscosity of the NaDES consisting of di and tri-protic acids. Airouywa et al., (2023) reported that higher molecular weight carboxylic acid-based NaDES possess higher viscosity; in the study of physicochemical properties of carboxylic acids based-NaDES, consisting of lactic acid, acetic acid, malic acid and citric acid as HBD while choline chloride as HBA, the HDB with di and tri protic acids possess higher viscosity compared to the monoprotic acids (acetic acid and lactic acids). The viscosity of choline chloride:lactic acid, choline chloride:acetic acid, choline chloride:maleic acid, and choline chloride:citric acid at 1:2 molar ratio were 20, 30, 6000, and 6800 cP, respectively. The high viscosity hinders the extraction efficiency due to the suppressed mass transfer within the system. Similarly, raspberry anthocyanins were extracted better in choline chloride-1,4-butanediol compared to other choline chloride-based eutectic mixtures (Xue et al., 2020). In a study when the chokeberry was extracted with hydroxypropyl-β-cyclodextrin in DES mixture, the extraction efficiency increased up to 3% of hydroxypropyl-β-cyclodextrin and decreased with further increase of hydroxypropyl-β-cyclodextrin. The initial increase could be due to the optimal "host-guest" interaction between anthocyanin and cyclodextrin to form an inclusion complex and stabilize the encapsulated anthocyanin. However, the decrease in anthocyanin extraction with increasing amount of hydroxypropyl-β-cyclodextrin can be directly related to the increased viscosity of the medium, which suppresses the anthocyanin extraction (Jovanović et al., 2023). Apart from these studies, several other studies (da Silva et al., 2020; Han et al., 2023; Zannou et al., 2020) have also reported a similar correlation between the viscosity and the extraction efficiency of anthocyanin.

Interestingly, when water is used as a co-solvent in a DES/NaDES mixture, the viscosity gets altered, resulting in significant changes in extraction efficiency. In addition, the use of water as the co-solvent increases the polarity of the DES/NaDES to increase the polar-polar interaction

between the DES/NaDES and anthocyanin (Vannuchi et al., 2022). Thus, the increased anthocyanin extraction efficiency of water is attributed to the decrease in the viscosity and increase in the polarity of the DES/NaDES. The addition of water reduces the viscosity of the DES/NaDES through the loss of the DES/NaDES supramolecular structure (Lanjekar et al., 2021). The reduction in viscosity favors high mass transfer and facilitates the extraction of anthocyanin. However, adding too much of water can disrupt the supramolecular network of DES and hence suppress the efficiency of extraction (Vannuchi et al., 2022). Extraction of anthocyanins from perilla leaves showed an increase of anthocyanin extraction yield from 470 to 508 mg /100 g DW when the water content was increased from 10 to 20%. However, when the water content was further increased to 30, 40, and 50% there was a steady decline in the anthocyanin extraction yield (Han et al., 2023). Mulberry anthocyanin extraction showed increased extraction yield when the water content in DES/NaDES increased from 10 to 30%. Afterwards, the extraction yield decreased as the water content increased to 40 and 50% (Guo et al., 2019). Although increasing the water content can favor the polarity of the DES/NaDES and a further decrease in the viscosity of the DES/NaDES mixture causes disruption in the molecular network among DES/NaDES, anthocyanin, and water matrix, thus impeding the extraction efficiency by weakening the bond between DES/NaDES and anthocyanin. Therefore, viscosity and the content of water play a crucial role in anthocyanin extraction.

3.4 Effect of solid-to-liquid ratio on the extraction of anthocyanins using DES/NaDES

The solid-to-liquid ratio is another important factor that affects the extraction of anthocyanins. The high amount of solvent aids mass transfer through a concentration gradient and provides a high collision probability for the liquid to penetrate the plant cells (MacLean et al., 2021; Xue et al.,

2020). When the liquid penetrates the cell wall matrix the anthocyanins can be easily extracted into the liquid due to the higher concentration gradient between solid and solvent. An increase in anthocyanin extraction from bran rice was observed when the liquid-to-solid ratio was gradually increased from 18.75 ml/g to 32.5 ml/g an no further increase was observed above 32.5 ml/g (Thakur et al., 2022). Similarly, raspberry anthocyanin extraction increased from 1.04 to 1.25 mg/g when the solid-to-liquid ratio was increased from 1:10 to 1:20, respectively. However, a further increase in the solid-to-liquid ratio (1:25 and 1:30) did not show a significant increase in the yield of anthocyanin (Xue et al., 2020). In contrast, purple perilla leaves the extraction yield of anthocyanin had a statistically significant increase from 365 to 450 mg/100g DW when the solid-to-liquid ratio increased from 1:4 to 1:10 (Han et al., 2023). These findings clearly suggest that the solid-to-liquid ratio does not always proportionately increase the extraction efficiency of anthocyanin and this could be attributed to the high content of liquid that can suppress the mass transfer from the solid phase .

Not only the aforementioned factors but also the integrative techniques can impact the extraction efficiency of anthocyanins. The ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are the two commonly used extraction techniques that are used in extracting anthocyanins from various raw materials (Guo et al., 2019). Using UAE, the anthocyanin extraction efficiency is increased through the acoustic waves, which will mechanically agitate the plant cell walls to rupture and at the same time create acoustic cavitation in the solvent medium. When the plant cell walls are ruptured, the anthocyanins become exposed to the DES/NaDES hence making the extraction process efficient. In addition, when acoustic cavitation is created the temperature in those regions increases and the viscosity decreases. The decreased viscosity enhances the feasibility of the solvent to penetrate the plant cell walls and extract the anthocyanin

(Jovanović et al., 2023; Thakur et al., 2022). Thus, these two effects synergistically work to enhance the anthocyanin extraction. Furthermore, in a UAE extraction method– the duration of the treatment, temperature, and the amplitude of the ultrasounds impact the extraction of anthocyanin. The Haskap berries showed maximum anthocyanin extraction when the ultrasound treatment was provided for 10 min at 75 °C (MacLean et al., 2021). Similarly, for black rice bran, the maximum anthocyanin extraction was achieved at 11.25 min at 21.31% amplitude of ultrasonication (Thakur et al., 2022). When raspberry anthocyanins were extracted with UAE– the maximum anthocyanin extraction was obtained at 210 W, 51 °C, and 32 min of power, temperature, and time, respectively (Xue et al., 2020). Depending on the cell matrix, the UAE parameters can vary and therefore, optimization studies have been carried out. Several other studies (Velásquez, Bustos, Montenegro, and Giordano (2021); Han et al. (2023); Alrugaibah, Yagiz, and Gu (2021)) have also optimized the extraction efficiency of anthocyanin from different sources.

MAE can be used as an alternative for UAE in anthocyanin extraction. The operating principle for MAE to extract anthocyanin is based on the ability of the microwaves to create rotational motion of the molecules, in a solid-liquid mixture, which can generate heat energy and accelerate the anthocyanin extraction. Three main factors determine the extraction efficiency of anthocyanin using microwave: microwave power, temperature, and time (Han et al., 2023). When purple perilla leaves were subjected to microwave extraction for anthocyanin– the extraction yield increased from 480 to 530 mg/100g DW at 40 and 60 °C, respectively while the microwave power was kept constant at 400 W. However, further increases in temperature to 70 and 80 °C, drastically decreased the anthocyanin yield. Similarly, when the microwave power was increased from 300 W to 400 W, the extraction yields steadily increased from 510 to 560 mg/100g DW and dropped significantly with further increase in microwave power to 500 W. Interestingly, when the purple

299 peril leaves were exposed in the microwave for more than 30 min the anthocyanin extraction yield
300 significantly dropped (Han et al., 2023). In contrast, when Hibiscus anthocyanin was extracted
301 using MAE, the total anthocyanin content increased with increasing microwave power, from 250
302 to 600 W, and time (50 to 150 s) (Kurtulbaş et al., 2020). A different study showed that the
303 maximum anthocyanin yield for purple sweet potatoes was obtained at 270 W microwave power
304 for 193 s (Evitasari et al., 2022). These studies clearly show that depending on the species, time,
305 temperature, and microwave power– the MAE of anthocyanin can vary. Most likely, when the
306 microwave treatment is given at a significantly high temperature for a prolonged period of time–
307 the anthocyanins can degrade– since they are thermally labile molecules. In summary, both, UAE
308 and MAE can be used as potential integrative techniques to enhance the extraction of anthocyanins.
309 More research is needed in terms of using NaDES/DES in a pressurized liquid extraction process
310 to investigate the extraction efficiency and stability of the extracted anthocyanin.
311
312 **4. Stability of anthocyanins in DES/NaDES**
313
314 Unlike conventional methods, the DES/NaDES extracted anthocyanins have shown better thermal
315 and/or storage stability. This is mainly attributable to the extensive intermolecular interactions that
316 occur between the biomolecules, such as anthocyanins and the DES/NaDES. Such intermolecular
317 interactions can suppress the movement of anthocyanins-like molecules to prevent oxidative stress
318 and maintain better stability. Moreover, unlike conventional solvents, the DES/NaDES can form
319 strong acylation with anthocyanin, which makes it stable at higher temperatures (Ruesgas-Ramón
320 et al., 2017). In light of such fact, many studies have investigated the stability of the anthocyanins
321 extracted using DES/NaDES.
322

When Roselle (*Hibiscus sabdariffa* L.) anthocyanin extracted in DES was exposed to temperatures 40 to 100 °C for 20, 40, 60, 80, and 100 min the thermal degradation instantly occurred at 100 °C due to the thermal instability of anthocyanin at very high temperatures. When these anthocyanins were stored at 20, 4, and -20 °C, the anthocyanins gradually degraded with time, at all three storage temperatures (Zannou et al., 2020). Similar observations were recorded with cyanidin of *Catharanthus roseus*; at 20 °C, the degradation was rapid compared to the other two temperatures (Yuntao Dai et al., 2016). The thermal stability of cyanidin from *C. roseus* was, also, studied by measuring the half-lives ($t_{1/2}$). The cyanidin had a greater than four-fold increase of $t_{1/2}$, in lactic acid: glucose DES/NaDES ($t_{1/2}$= 277.3 min) compared to ethanol ($t_{1/2}$= 72.2 min). The $t_{1/2}$ of blueberry anthocyanins also showed a similar trend where in DES the $t_{1/2}$ was 141.6 min compared to conventional organic solvent, where $t_{1/2}$ was 45.1 min (da Silva, Smaniotto, Costa, Baranzelli, Muller, Somacal, Monteiro, Vizzotto, Rodrigues, Barcia, et al., 2021). The Brazilian berry anthocyanins extracted with choline chloride: propylene glycol and choline chloride: malic acid showed longer $t_{1/2}$ for choline chloride: malic acid ($t_{1/2}$= 9.8 hr) as opposed to chloride: propylene glycol ($t_{1/2}$= 5.7 hr), at 60 °C. The affinity of malic acid to anthocyanin could be related to its functional groups (Benvenutti et al., 2022). The same study also reported the anthocyanin stability in terms of thermodynamic parameters: The enthalpy change (ΔH) for choline chloride: malic acid was comparatively higher than choline chloride: propylene glycol. The significantly high ΔH is due to the high activation energy (E_a) of choline chloride: malic acid (77.6 kJ/mol), which clearly shows that the complex formed between anthocyanin and choline chloride: malic acid is stronger and requires more energy to break the bonds for degradation. Interestingly, both, choline chloride: malic acid and choline chloride: propylene glycol showed negative values for change in entropy (ΔS) due to the highly ordered structure that forms between anthocyanin and the DES mixture. The ΔS was highly negative for choline chloride: malic acid (ΔS at 60 °C was -42.7 J/mol)

compared to choline chloride: propylene glycol (ΔS at 60 °C was -75.6 J/mol) because of the highly ordered structure that forms between anthocyanin and choline chloride:malic acid – due to the intense intermolecular interactions. Overall, the anthocyanins extracted using DES have clearly shown increased stability towards time-dependent degradation.

5. Extraction of carotenoids using DES/NaDES

Carotenoids are secondary metabolites found in the chloroplast or chromoplast of fruits, vegetables, and microorganisms. Depending on the chemical structure, the carotenoids can be classified as: (1) carotenes, the carotenoids that are composed of hydrocarbon (β-carotene, lycopene); (2) xanthophyll, an oxygenated derivative of carotene (lutein, zeaxanthin, astaxanthin) (Yu et al., 2022). The chemical structure of major carotenoids is illustrated in **Figure 4**. Since these carotenoids are hydrophobic and lipophilic, the extraction is mostly facilitated by organic solvents. However, recently, deep eutectic solvents have become promising solvents for extracting carotenoids (Koutsoukos et al., 2019). The increased interest in carotenoid extraction using a green solvent is owed to its substantial health benefits– which in turn is beneficial in, both, culinary, food, and nutraceutical applications.

5.1 Extraction of total carotenoids

Carotenoids from *Lycium barbarum*, a berry with various medicinal properties, showed better extraction yield when choline chloride and malonic acid were used at a 1:1 ratio. However with increasing malonic acid concentration, the extraction efficiency declined. The optimal ratio of choline chloride to malonic acid provided a favorable medium for carotenoids to be extracted. Change in viscosity with increasing concentration of malonic acid would have been the major

reason for the decreased efficiency of carotenoids (Z. Yu et al., 2022b). A deep eutectic solvent system with choline chloride and tartaric acid with ultrasound-assisted extraction and microwave-assisted extraction showed better carotenoid extraction for apricot pulp. For example, the carotenoid extraction with conventional solvent-coupled with UAE was 11.5 mg β-carotene/100g DS, whereas with choline chloride-tartaric acid-UAE, the carotenoid extraction increased to 41.3 mg β-carotene/100g DS. Similarly, the carotenoid extraction was higher for choline chloride-tartaric acid- MAE (26.7 mg astaxanthin/100g DS) compared to MAE with conventional solvents (23.9 mg astaxanthin/100g DS). The increased carotenoids with DES could be attributable to the strong intermolecular interaction between the carotenoids and the DES complex (Koutsoukos et al., 2019). Orange peels are rich sources of carotenoids. When orange peel carotenoids were extracted with various DES systems, menthol-camphor, menthol-eucalyptol, and lauric acid-octanoic acid showed high yields. Compared to other DES systems used in this study, these three solvents showed higher extraction due to the hydrophobicity. The higher extraction efficiency of these three solvents is also reflected in the higher oxygen radical absorbance capacity assay (ORAC)– mainly owed to the radical scavenging potential of these hydrophobic DES. Nevertheless, in terms of storage stability, the carotenoids showed better stability with menthol-eucalyptol, which could be mainly due to the higher thermal stability of menthol-eucalyptol (Viñas-Ospino et al., 2023). Similarly, another study that screened 68-DES for carotenoid extraction from orange peel showed better extraction with menthol: camphor coupled with UAE. Interestingly, crude palm oil that has ≈1% carotenoids showed the highest extraction yield with menthol: lactic acid at 1:1.5 (212 ppm) compared to menthol: acetic acid at 1:1.5 (150 ppm) (A. Viñas-Ospino et al., 2023b). In contrast to all these studies, buriti fruit (*Mauritia flexuosa*) did show an increase in carotenoid when various choline chloride-based solvents (choline chloride with ethylene glycol, glycerol, glycerol-xylitol, glycerol-PEG) were used for extraction. This

observation could be related to the weak interaction between buriti carotenoids and the DES system due to their incompatible functional groups (Leite et al., 2021). In summary, high carotenoid extraction is favored when the DES forms strong hydrophobic and/or lipophilic interactions with different functional groups found in carotenoids.

5.2 Extraction of carotenes

Carotenes are a class of carotenoids that mainly include β-carotene and lycopene. The β-carotene is mainly responsible for the orange, red, and orange-red color of fruits, vegetables, and flowers (J. Yu et al., 2022). Due to the hydrophobicity of the β-carotene, the extraction efficiency can be increased by using fatty acids such as HBD and HBA. In a study, where medium-chain fatty acids and short alkyl-chain fatty acids were used as HBD and HBA, respectively, the extraction of β-carotene increased when ternary DES/NaDES was used. In addition, with ternary DES, the highest extraction efficiency of approximately ≈100% was achieved when short (C_9) and medium chains (C_{10} and C_{11}) were used at a 2:1:1 ratio. The tuning of fatty acid-based DES composition can change the extraction efficiency of β-carotene by varying the degree of hydrophobic interactions (Li et al., 2019). By the same token, the extraction of β-carotene from pumpkin showed high extraction efficiency in a caprylic acid: capric acid eutectic mixture at 3:1. The higher extraction efficiency of this eutectic mixture, compared to other mixtures used in this study, was due to the increased solubility of the β-carotene in the eutectic mixture used. For example, caprylic acid: capric acid and menthol: lauric acid mixture showed 200.8 and 97.2 μg/mL of β-carotene solubility, respectively and this was translated to high extraction efficiency in caprylic acid: capric acid 96.7 μg/mL and lowest extraction efficiency in menthol: lauric acid 81.4 μg/mL. Interestingly, switching the polarity from hydrophobic to hydrophilic aided in recovering the extracted β-

carotene in the DES mixture. When water was added to the β-carotene extracted by DES a two-phase separation was obtained due to the immiscibility of the fatty acid-based DES and water. However, the addition of a mild base (NH_4OH) to this mixture resulted in a homogenous solution, which eventually precipitated the β-carotene that was extracted in the DES which resulted in a higher yield of recovery which was about ≈ 90% (Stupar et al., 2021). Extraction of β-carotene from tomato pomace using conventional solvents and eutectic mixture showed a statistically significant difference in the extraction efficiency. The acetone: n-hexane (conventional solvent) and ethyl acetate: ethyl lactate (eutectic mixture) showed extraction efficiency of 2117.6 and 1510.2 μg/g, respectively (Lazzarini et al., 2022). When β-carotene was extracted from *Phaffia rhodozyma* yeast using ionic liquids and deep eutectic solvents, significantly high β-carotene was obtained with choline chloride: butanoate at 1:2 (Mussagy et al., 2022). Overall, various studies have proved the efficiency of DES in the extraction of β-carotene from different sources.

Lycopene, is another important carotenoid that is majorly found in tomatoes. One study with different eutectic mixtures such as capric acid: menthol, capric acid: thymol, capric acid: lauric acid, lauric acid: menthol, and lauric acid: thymol showed highest lycopene extraction of 7.5 mg/100g FW with capric acid: lauric acid at 1:2 ratio. The lowest yield 1.1 mg/100g FW was obtained with capric acid: menthol at 1:2 ratio. This could be attributable to the density and the flow behavior index of the eutectic mixtures. In the case of capric acid: menthol, the density was slightly higher than capric acid: lauric acid, which was 0.989 and 0.986, respectively. This was also translated to the high flow behavior index of capric acid: menthol (1.2), compared to capric acid: lauric acid (1.0). Hence, less mass transfer in a relatively high-density eutectic mixture could hinder lycopene extraction (Kyriakoudi et al., 2022). Tomato pomace, one of the by-products of the tomato industry, showed high lycopene extraction, 1446.6 μg/g, in menthol: lactic acid (8:1)

eutectic system when it was integrated with UAE at 70 °C for 10 min at 120 mL/g solvent-to-solid ratio (Celeste Lazzarini et al., 2022). Another study showed that the lycopene extracted using ethyl acetate: ethyl lactate accounted for 27.4 μg/g (Silva et al., 2019). The significant difference in the lycopene extract in these two studies can be related to the UAE that facilitated the lycopene extraction. In a fatty acid-based eutectic mixture, the highest lycopene of 90% was obtained when the eutectic mixture was composed of 1-nonaoic acid: n-decanoic acid: undecanoic acid at a 2:1:1 ratio (Li et al., 2019). The studies reported on lycopene extraction from tomato and tomato by-products have clearly shown that deep eutectic solvents are efficient solvent systems in extracting lycopene.

5.3 Extraction of xanthophyll

To date, astaxanthin is the highly explored xanthophyll for eutectic extraction. The astaxanthin is mainly found in microorganisms and also accumulates in the shells of crustaceans, which consume microalgae (Rodrigues et al., 2020). Extraction of astaxanthin from crab shells using a terpene-based eutectic mixture showed the extraction yield was comparable to Soxhlet method using acetone and eutectic mixture of menthol-myristic acid at 8:1 ratio, treated at 60 °C for 24-hours (Rodrigues et al., 2020). In contrast, the high astaxanthin extraction was obtained with menthol-myristic acid at an 8:1 ratio for shrimp, mussels, and *Haematococcus pluvialis* (Rodrigues et al., 2020), compared to the conventional acetone-based Soxhlet method. When astaxanthin was extracted from shrimp shell waste to utilize in bioactive films the maximum yield of 69.08 μg/g of astaxanthin was obtained using choline chloride: lactic acid mixture at 1:1.02 ratio. The microalgae *H. pluvialis* treated with oleic acid-terpene (thymol, menthol, and geraniol) showed maximum extraction efficiency in the oleic acid-geraniol eutectic mixture due to the increased hydrophobic

interaction with astaxanthin. However, the stability of the extracted astaxanthin was high in the oleic acid-thymol mixture due to the enhanced free radical scavenging ability of thymol, compared to menthol and geraniol (Rodrigues et al., 2020). Another study with *H. pluvialis* showed maximum extraction with choline chloride-butanoate (Pitacco et al., 2022). Astaxanthin extraction from Gazami crab (*Portunus trituberculatus*) with methyl triphenyl phosphonium bromide-glycerin eutectic mixture at 1:4 ratio integrated with 65 W ultrasonic power for 90 min resulted in 39.37 μg/g of astaxanthin of *P. trituberculatus* waste (Lee & Row, 2016). Similarly, other studies have also shown efficient extraction of astaxanthin using eutectic mixtures from Brazilian shrimp (*Litopenaeus vannamei*) (Santos et al., 2021) and shrimp by-products (Chandra Roy et al., 2021; Zhang et al., 2014). All these studies have shown that due to the hydrophobicity of astaxanthin, the extraction efficiency is high in hydrophobic eutectic systems, such as fatty acid-terpene eutectic mixture.

6. Toxicology of common DES used in pigment extraction

Deep eutectic solvents (DES) are considered "green solvents" and "solvents of the 21st century" (Paiva et al., 2014). When the components of DES are obtained from natural sources, the DES forms a subclass that is known as Natural Deep Eutectic Solvents (NaDES). Both DES and NaDES are considered benign, less-toxic, biodegradable, and cost-effective (Hayyan et al., 2013; Wen et al., 2015; Zhao et al., 2015). Thus, these solvents are currently replacing conventional organic solvents and ionic liquids for respective applications. Nevertheless, the toxicity and safety of the DES and NaDES are debatable to date. Thus, the following section will cover the cytotoxicity and biodegradability (ecotoxicity) studies conducted on the common DES and NaDES that are used for the extraction of anthocyanins and pigments.

The commonly used eutectic solvents for anthocyanins and pigments are mainly based on choline chloride and terpene. The choline is a major precursor for the synthesis of cell membrane and its salt form, choline chloride, is considered safe as well (Martínez et al., 2022). The terpenes are secondary metabolites of plants and hence are GRAS. However, when the NaDES are synthesized with other natural metabolites such as sugars (glucose, fructose), sugar alcohols (xylitol, maltitol), organic acids (malonic acid, oxalic acid), amides (urea)– the degree of toxicity changes due to the intermolecular interactions (Rodrigues et al., 2020). These interactions also translate to various physicochemical properties that can dictate the toxicity of the compounds. Studies (Hayyan et al., 2013; Radošević et al., 2018) have shown that NaDES are more toxic compared to their individual components. For example, the individual components (choline chloride, glycerin, ethylene glycol, triethylene glycol, and urea) showed no toxicity with *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, but they did exert significant toxicity when the eutectic mixture was synthesized based on choline chloride (Radošević et al., 2018). Similarly, when citric acid was treated on different gram-positive and gram-negative bacteria, the toxicity was less but the citric acid in a eutectic mixture manifested high toxicity (Radošević et al., 2018). These observations can be attributed to the: (1) increase in viscosity of DES and/or NaDES, which can hinder the oxygen availability and hence cause oxidative stress; (2) penetration of the eutectic mixture through the cell membranes, which in turn can cause destabilization of the cell membrane matric through "Hofmeister colloidal principle"; (3) change in the pH of the eutectic mixture, which can disrupt the biochemical functions in the cells; (4) addition of water as the ternary component in the eutectic mixture, which can not only change the supramolecular structure of the eutectic mixture but also the interaction of the eutectic mixture with the cell surface (Lomba et al.,

2021; Radošević et al., 2018; Sanchez-Fernandez et al., 2021). Due to these reasons, the DES/NaDES have shown varying degrees of cytotoxicity and ecotoxicity.

The cytotoxicity quantified by EC_{50} values showed that the choline chloride-oxalic acid had significantly high toxicity for HeLa and MCF-7 cells and these were quantified as 2.48 mM and 4.19 mM, respectively. In addition, the same study showed that the choline chloride-urea was toxic to MCF-7 cells and not to HeLa or HEK293T cells (Hayyan et al., 2013). The citric acid-trehalose eutectic mixture at 12.24 μM concentration– a potential eutectic system for anthocyanins and/or carotenoids, showed no cytotoxic effects on Zebrafish (Ferreira et al., 2022). Interestingly, when choline chloride, tetramethylammonium chloride, and tetrabutylammonium chloride-based eutectic systems were studied on human skin cells (HaCaT) and tumor melanocytes (MNT-1), the cell viability dramatically decreased with increasing concentration of the eutectic mixture (Macário et al., 2019). Especially with tetrabutylammonium chloride-urea mixture on HaCaT cells, the decrease was significant compared to tetrabutylammonium chloride-hexanoic acid, butanoic acid, 1-propanol, and ethylene glycol. In contrast, for MNT-1 cells, significant cell viability was seen with tetrabutylammonium chloride-1-propanol. The selectivity of the eutectic mixture in terms of decreasing the cell viability could be translated to the potential aggregation that can be caused between the cell membrane and the eutectic solvent, which suppressed the tolerance of cells for survival (Macário et al., 2019). The terpene-based eutectic solvents that are commonly used for carotenoid extraction have shown varying degrees of cytotoxicity with Caco-2 cells. For example, the astaxanthin extracted from crab shells with terpenes (perillyl alcohol, camphor, menthol, eucalyptol) and myristic acid showed EC_{50} between 0.5 to 1.1 mg/mL against Caco-2 cells. Similarly, shrimp shells, mussels, and *H. pluvialis* extracted with DES comprising of menthol: myristic acid showed EC_{50} of 1.5, 1.8, and 3.3 mg/mL against Caco-2 cells, respectively

(Rodrigues et al., 2020). Strikingly, another study with mice exposed to a choline chloride-urea eutectic mixture showed cytotoxicity due to the elevated levels of ammonium which caused oxidative stress due to oxygen and nitrogen (Jung et al., 2021). All the aforementioned studies show that the varying degree of cytotoxicity depends not only on the nature of the eutectic mixture but also on the type of cell lines used to study the cytotoxicity.

While cytotoxicity measures the protection of human health, ecotoxicity is imperative to monitor environmental protection when using chemical solvents. In light of such fact, eutectic solvents are also studied for ecotoxicity in both terrestrial and aquatic systems followed by their potential to biodegrade. A concentration of 0.001 M of the citric acid-trehalose eutectic system was introduced to water containing Zebrafish. The fish showed no toxicity when exposed to such an aquatic environment (Ferreira et al., 2022). When different choline chloride and choline acetate-based eutectic solvents with acetamide, urea, glycerol, and ethylene glycol at 0.01 M were introduced in the freshwater ecosystem, the survival times of hydra was highly dependent on the nature of the eutectic mixture (Wen et al., 2015). Overall, the survival time was higher for the choline acetate-based eutectic solvent, compared to the choline chloride-based mixture; higher survival time (12 hours) were seen for choline acetate-acetamide and choline acetate-urea. However, the negative growth of the tentacles in hydra occurred within a few hours– demonstrating the toxicity from the eutectic mixture. The ecotoxicity studied on *Aliivibrio fischeri*, which is a model animal used to study aquatic toxicity due to its luminescence effect, showed that all the eutectic mixtures (choline chloride, tetramethylammonium chloride, tetrabutylammonium) showed ecotoxicity due to the ability of these eutectic mixtures to penetrate the cells, mainly the cytoplasm, and cause disruptions in the cellular process (Giner et al., 2020). In terms of the terrestrial system, the toxicity of the eutectic mixture was studied based on the root growth of *Allium sativum*. Compared to control

samples (4.5 cm), all the other eutectic systems and their individual components showed truncated growth of the roots. Mainly, the choline chloride-ethylene glycol had the lowest growth, compared to others. In addition, the root tip cells were deformed, irrespective of the nature of the eutectic solvent (Wen et al., 2015). Similar observations were also reported for T*riticum aestivum* species (Nejrotti et al., 2022). These observations could be supported by the fact that eutectic mixtures– due to their strong intermolecular interactions– translated from the supramolecular structure, can cross the cell surfaces and penetrate the subcellular levels to disrupt and/or attenuate the cell growth. The biodegradability of the choline chloride and choline acetate studied against sodium benzoate showed significantly lower biodegradability. The biodegradability over 14 days for sodium benzoate, choline chloride-urea, and choline acetate-urea were 90, 78, and 40%, respectively and the lowest biodegradability (25%) was observed for choline chloride-ethylene glycol. Furthermore, the choline chloride-based eutectic mixture was more biodegradable than the choline acetate-based eutectic mixtures, which could be due to the different degradation mechanisms carried out by different soil microorganisms (Wen et al., 2015). In summary, the ecotoxicity studies have shown that the eutectic mixtures are toxic to a certain extent for, both, aquatic and terrestrial systems and show considerable amounts of biodegradability– although these mixtures cannot be labelled as "readily biodegradable".

7. Bioavailability of pigments extracted using DES

DES possess high bioavailability, bio-accessibility, and bio-stability due to the strong hydrogen bonding network that is formed between DES and the bioactive compounds. However, only a few studies on the bioavailability of the pigments extracted with DES have been reported. A study by Zannou et al. (2022) reported the high bioaccessbility and bio-stability of anthocyanin extracted

with choline chloride: glycerol, by simulating the in vitro gastrointestinal model to mimic the physiological in vivo digestion. The anthocyanin investigated, in this study, were cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and cyanin chloride and the bioavailability of these anthocyanins accounted for 71.9 ±0.5, 77.3 ±0.6, 80.2 ±0.7, and 91.0 ± 1.0%, respectively. In general, the high pH in the intestinal environment biotransforms anthocyanin by reducing its bioavailability. However, in this study, the bioavailability of the anthocyanins was 70 to 90% and this is attributable to the ability of the choline chloride-glycerol to protect the functional groups of anthocyanin; the extensive hydrogen bond network between the DES (choline chloride-glycerol) and the anthocyanin (cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside, and cyanin chloride) prevents the biotransformation at alkaline pH. In a different study, the bioavailability of blueberry anthocyanin in rats was compared between DES (choline chloride-glycerol-citric acid) and organic solvent (methanol-water-formic acid). When an equal amount of anthocyanin extracted from DES and organic solvent was administered to the rats, the bioavailability of anthocyanin was 140% more in DES-based anthocyanin, compared to organic solvent-based anthocyanin (da Silva et al., 2021). In addition, the DES-extracted anthocyanin showed a biphasic profile in the gastrointestinal absorption confirming the intactness of anthocyanin– when DES is used as the extraction solvent. Therefore, the in vitro and in vivo digestion studies have shed some light on the bioavailability, bio-accessibility, and bio-stability of the DES-extracted pigments. It is expected that more research in this direction will unfold in the coming years to provide a better understanding of the bioaccessibility and bioavailability of the NaDES-based anthocyanin extracts.

8. Recovery of anthocyanin and carotenoid from DES/NaDES

The recovery of carotenoid and anthocyanins from DES/NaDES is a challenging process because these solvents possess low vapor pressure and can form strong molecular interactions between the extracted compounds of interest, making the recovery process tasking. Several methods have been described for removing target compounds from DES/NaDES which include, (1) liquid-liquid extraction by including additional solvents, (2) antisolvent by addition of a solvent with different polarity from the extraction solvent (3) solid phase extraction by using high-adsorbent resin and chromatography (Grillo et al., 2020). de Souza et al. (2023) recovered 100% of the grape pomace anthocyanins from DES using solid-phase extraction with a high-adsorbent resins. The resin can also be reused from 1-8 cycles with an average recovery of 96±1% of grape pomace anthocyanin. While the recycle yield of DES was 96±1% at the first recycle. Similarly, Panić et al., (2019) reported the recovery of anthocyanins from grape pomace from NaDES using the solid-phase extraction with macroporous resin. A total of 70.34% anthocyanins were recovered and 94.78% NaDES recovered. In the recovery of anthocyanins from blueberry peel extract using absorption chromatography and macroporous resin reported by Grillo et al., (2020). The results revealed that the recovery of anthocyanins and NaDES were 72.55 and 79.48%, respectively. Furthermore, the recovery of carotenoid from pumpkins using switchable NaDES was reported by Stupar et al., (2021). It was proposed that the possibility of separating carotenoids from hydrophobic NaDES was based on the potential of the NaDES to switch its polarity by the addition of water and a weak base to precipitate the carotenoid from the carotenoid NaDES-based extract. β-carotene was the major carotenoid precipitate followed by β-cryptoxanthin with a total recovery of 52.25 and 38.04%, respectively. The possibility of recovery of anthocyanins and carotenoids from DES/NaDES and the ability to reuse the DES and other materials like the microporous resin makes the entire process economical and sustainable.

9. Future perspectives and current limitations of DES in anthocyanin and carotenoid extraction

Over the past decade, DES and NaDES have gradually replacing conventional solvents for extracting bioactive compounds. In alignment with such change, the extraction of anthocyanins and carotenoids– which have significant applications in food and nutraceutical industries has become a focus of attention.

One of the major challenges in using eutectic mixtures is to enhance the extraction efficiency and recover the extracted component within a short period of time. In-situ preparation of the eutectic mixtures and switchable eutectic solvents are investigated for the enhanced purification, separation, rapid extraction, and recovery of eutectic mixture (Ahmadi et al., 2023; Stupar et al., 2021; Zhang et al., 2023). In-situ preparation of eutectic mixtures is robust, compared to the traditional eutectic mixture preparation; traditional preparation of eutectic mixture involves either heating the eutectic mixture to a higher temperature for a longer period of time or freeze drying the components in the eutectic mixture over few days. In contrast, an in-situ formation involves the simultaneous complex formation of HBA and HBD in the sample mixture, where the HBA-HBD complex can instantly bind to the components for rapid extraction (Ahmadi et al., 2023; Niu et al., 2023). In terms of switchable eutectic solvents, these employ the changes in pH, temperature, polarity, conductivity, density, and solubility. The flexibility to tailor the solvent system during the extraction process makes eutectic solvents a versatile mixture (Zhang et al., 2023). The use of a switchable solvent by changing the polarity has shown the efficient recovery of β-carotene from pumpkin seeds (Stupar et al., 2021). In addition, switching the polarity to fractionate the biomasses

has also emerged in recent years. Although the switchable eutectic solvents are promising, the research in this area is yet to be explored.

A recent investigation into DES/NaDES has revealed new findings in the field of biocatalysis. The eutectics can act as, both, solvent and substrate (2-in-1) to increase the atomic efficiency of the biocatalytic process (Pätzold et al., 2019). One such highly explored biocatalytic system is lipase-based biotransformation– driven by the stereoselectivity of the DES/NaDES. Such an approach can be translated to pigment extractions because mostly the first step of pigment extraction involves an enzymatic reaction to disintegrate the pigments from the plant cell wall (Mi ˛ekus et al., 2019). Under such circumstances, the Des/NaDES can be used as a co-solvent and substrate to increase the efficiency of pigment extraction. Nevertheless, the molecular insights of such transformations are yet to be explored.

Interestingly, the use of eutectic solvents for extraction in various applications involves optimizing eutectic mixtures based on their molar ratio, solid-to-solvent ratio, pH, and thermal stability. However, the process of screening eutectic solvents is time-consuming and involves significant costs. Therefore, a recent advance in such area is the use of a Conductor-like Screening Model for Real Solvents (COSMO-RS) to identify the best solvent for enhanced and rapid extraction of bioactive compounds (Hayyan et al., 2016; Panić et al., 2021). Although the concept of COSMO-RS is not new, the use of this software for eutectic solvent extraction can be considered relatively new. In two recent studies, the eutectic solvents to extract bioactive components from blueberry (da Silva, Smaniotto, Costa, Baranzelli, Muller, Somacal, Monteiro, Vizzotto, Rodrigues, & Barcia, 2021) and grape pomace (Panić et al., 2021) were studied using COSMO-RS. Unlike manual screening, the use of this computational model aided in screening multiple solvents to find

the suitable solvent for enhanced extraction. This technique can also be further extended to create ready-to-use eutectic solvents, which facilitates the selection of an eutectic mixture to extract the bioactive component of interest. Considering the aforementioned recent advances in extracting bioactive components using eutectic mixtures, it is clear that the eutectic solvents are promising for the extraction of anthocyanins and carotenoids.

Conflict of interest

All authors declare no conflicts of interest. For the purpose of open access, the author, Ali Ali Redha, has applied a 'Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising'.

Author contribution

Jennifer Osamede Airouyuwal: Writing - original draft, Revising the manuscript, data compilation

Nilushni Sivapragasam: Writing - original draft, Revising the manuscript, data compilation

Ali Ali Redha: Visualization, Writing - review & editing, data compilation, designing figures.

Sajid Maqsood: Conceptualization, Writing - review & editing, Project administration, Supervision.

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List of tables

1032 Table 1: Summary of studies on anthocyanin extraction from natural sources using DES/ NaDES

1033 and the extraction parameters used.

Natural sources	**Extracting solvent composition**	**Extraction method**	**Extraction yield of anthocyanin**	**Reference**
Bilberry (*Vaccinium myrtillus* L.)	Choline chloride: sorbitol (1:1)	UAE	2.0 mg CGE/g DW	Jovanović et al. (2022)
Chilean berries (*Luma chequeen*)	Lactic acid: glucose (8:1)	UAE	3.3 mg CGE/g DW	Velásquez, Bustos, Montenegro, and Giordano (2021)
Black rice (*Chakhao ambu*) bran	Lactic acid: fructose (1:1)	UAE	32.2 mg/L	Thakur, Gupta, Dhar, Deka, and Das (2022)
Haskap berry (*Lonicera caerulea* L.)	Citric acid: maltose (1:1)	UAE	21.2 mg/g DW	MacLean, Silva, Jiao, and Brooks (2021)
Perilla leaves	Choline chloride: ethylene glycol: lactic acid (1:1:1)	MUAE	6.2 mg/g DW	Han et al. (2023)
Cranberry (*Vaccinium macrocarpon*) pomace	Lactic acid: glucose (5:1)	UAE	1.6 mg/g DW	Alrugaibah, Yagiz, and Gu (2021)

Wine lees	Choline chloride: malic acid (1:1)	UAE	5.5 mg/g DW	Bosiljkov et al. (2017)
Purple sweet potato (*Ipomoea batatas* L.)	Citric acid: ethylene glycol (1:1)	MAE	311.6 mg/L	Evitasari, Rofiqoh, Damayanti, and Chusna (2022)
Jussara (*Euterpe edulis*) fruit pulp	Choline chloride: xylitol (1:1)	UAE	14.9 mg/g DW	Vannuchi, Braga, and De Rosso (2022)
Blackberry (*Rubus* spp)	Choline chloride: acetic acid (1:2)	UAE	1.2 mg CGE/g DW	Zannou and Koca (2022)
Grape (*Vitis vinifera*) skin	Citric acid: maltose (4:1)	UAE	42.0 mg/g DW	Jeong et al. (2015)
Mulberry (*Fructus Mori*)	Choline chloride: citric acid: glucose (1:1:1)	HSH-CBE	6.1 mg/g DW	Guo et al. (2019)
Blueberry (*Vaccinium* spp)	Choline chloride: glycerol: citric acid (0.5:2:0.5)	Heating in boiling water bath	3.6 mg CGE/g	da Silva et al. (2020)
Chokeberry (*Aronia melanocarpa*)	Choline chloride: lactic acid (1:2)	UAE	6.0 mg CGE/g DW	Jovanović et al. (2023)
Raspberry (*Rubus idaeus* L.)	Choline chloride: 1,4-butanediol (1:3)	UAE	1.4 mg/g DW	Xue, Tan, Li, Tang, and Cai (2020)

Brazilian berry (*Myrciaria cauliflora*)	Choline chloride: malic acid (1:1)	PLE	62.9%	Benvenutti, Zielinski, and Ferreira (2022)
Roselle (*Hibiscus sabdariffa* L.)	Sodium acetate: formic acid (1:2)	UAE	10.6 mg D3S/g DW	Zannou, Koca, Aldawoud, and Galanakis (2020)
Catharanthus roseus	Lactic acid: glucose & Choline chloride:1,2 propanediol	UAE	Not specified	Dai, Rozema, Verpoorte, and Choi (2016)
Roselle (*Hibiscus sabdariffa* L.)	Citric acid: ethylene glycol (4:1)	MAE	3.0 mg C3G/g DW	Kurtulbaş, Pekel, Bilgin, Makris, and Şahin (2020)

Abbreviations: CGE: cyanidin-3-O-glucoside equivalents, D3G: delphinidin-3-sambubioside equivalents, UAE: Ultrasound-Assisted extraction, MAE: Microwave-Assisted Extraction, MUAE: Microwave-Ultrasound-Assisted Extraction, PLE: Pressurized Liquid Extraction, HSH-CBE: High-speed homogenization and Cavitation-burst extraction.

Table 2: Summary of studies on carotenoid extraction from natural sources using DES/ NaDES and the extraction parameters used.

Natural sources	Extracting solvent composition	Extraction method	Extraction yield of pigments	Reference
Total Carotenoids				
Lycium barbarum	Choline chloride: malonic acid (1:1)	UAE	Peak area: Zeaxanthin (34,000) Zeaxanthin dipalmitate (118,00000)	Yu et al. (2022)
Shrimp head	Choline chloride: tartaric acid (2:1)	MAE	26.7 (mg astaxanthin/100 g DS)	Koutsoukos, Tsiaka, Tzani, Zoumpoulakis, and Detsi (2019)
Apricot pulp	Choline chloride: tartaric acid (2:1)	MAE	76.1 (mg of β-carotene/100 g DS)	Koutsoukos et al. (2019)
Buriti fruit (*Mauritia flexuosa* L.)	Choline chloride-based DES as co-solvent	Conventional extraction	Buriti pulp: 1006 (mg/100 g DW) Buriti peel: 1043 mg/100 g DW)	Leite et al. (2021)
Orange peel	Menthol: Eucalyptol (1:1)	Homogenization and stirring	150 (mg/ 100 g FW)	Viñas-Ospino, Panić, Bagović, et al. (2023)
Carotene (β-carotene and lycopene)				
Phaffia rhodozyma	Choline chloride: butanoate (1:2)	Stirring, centrifugation, and filtration	45% yield β-carotene	Mussagy et al. (2022)
Fruit juices (watermelon, grape, tomato, guava)	Fatty acid: C9:C10:C11 (2:1:1)	Centrifugation and filtration	>50% yield of β-carotene and lycopene	(Li, Zhao, Tian, Yang, & Li, 2019)
Pumpkin	Fatty acid: C8:C10 (3:1)	UAE	151.4 (µg/ mL β-carotene)	Stupar et al. (2021)
Tomato pomace	Ethyl acetate: ethyl lactate (30:70 v/v)	Stirring, centrifugation, and filtration	3950.1 (µg/g β-carotene) and 75.9 (µg/g lycopene)	Lazzarini et al. (2022)
Xanthophyll (astaxanthin)				
Haematococcus pluvialis	Thymol: oleic acid (1:1)	Stirring, centrifugation	60% (yield in 6 hr)	Pitacco et al. (2022)

Shrimp by-products	Choline chloride:1,2-butanediol (1:2)	UAE	146 (µg/g)	Zhang, Tang, and Row (2014)
Shrimp residue	Choline chloride: glycerol (1:2)	UAE	32.7 (µg/g)	Santos et al. (2021)
Portunus trituberculatus waste	Methyl triphenyl phosphonium bromide:1,2-butanediol (1:4)	UAE	47.3 (µg/g)	(Lee & Row, 2016)
Shrimp waste	Choline chloride: lactic acid (1:2)	UAE	60.1 (µg/g)	(Chandra Roy et al., 2021)
Phaffia rhodozyma	Choline chloride: butanoate (1:2)	Stirring, centrifugation, and filtration	≈ 50% yield	(Mussagy et al., 2022)
Brown crab shell	Menthol: myristic acid (8:1)	Stirring and centrifugation	9.5 µg/g	(Rodrigues et al., 2020)

Abbreviations: UAE: Ultrasound-Assisted extraction, MAE: Microwave-Assisted Extraction.

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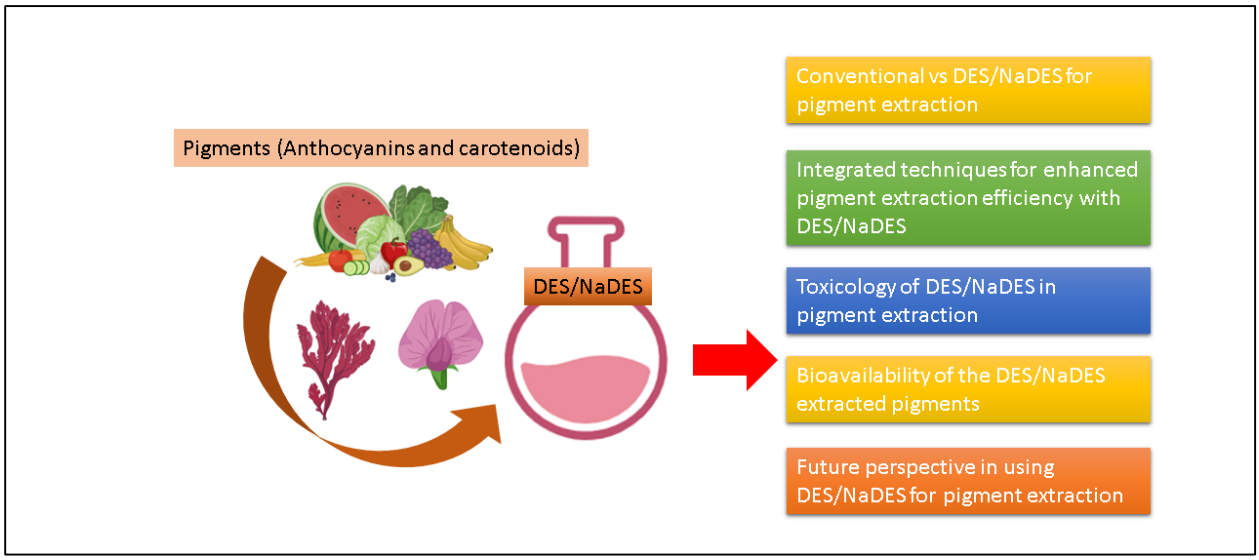
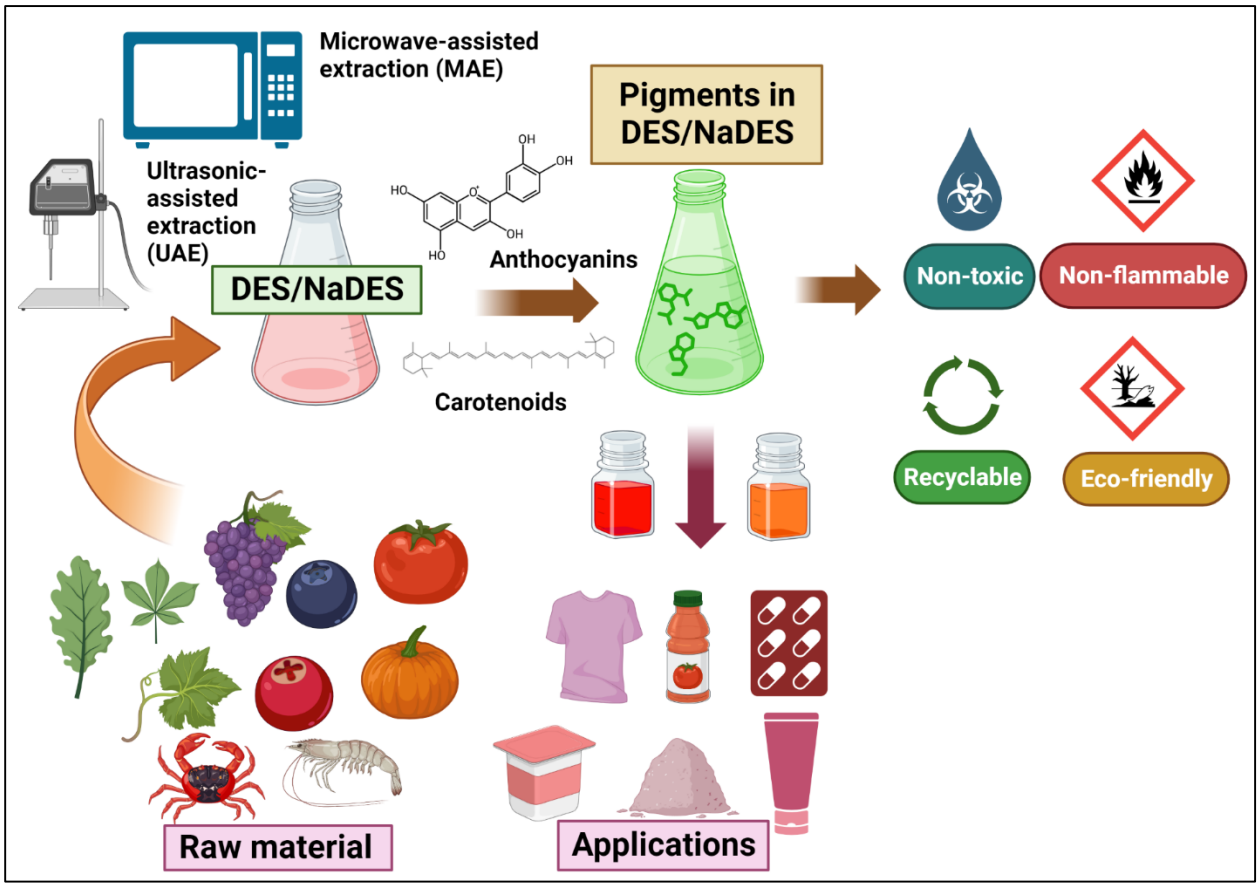


Graphical abstract



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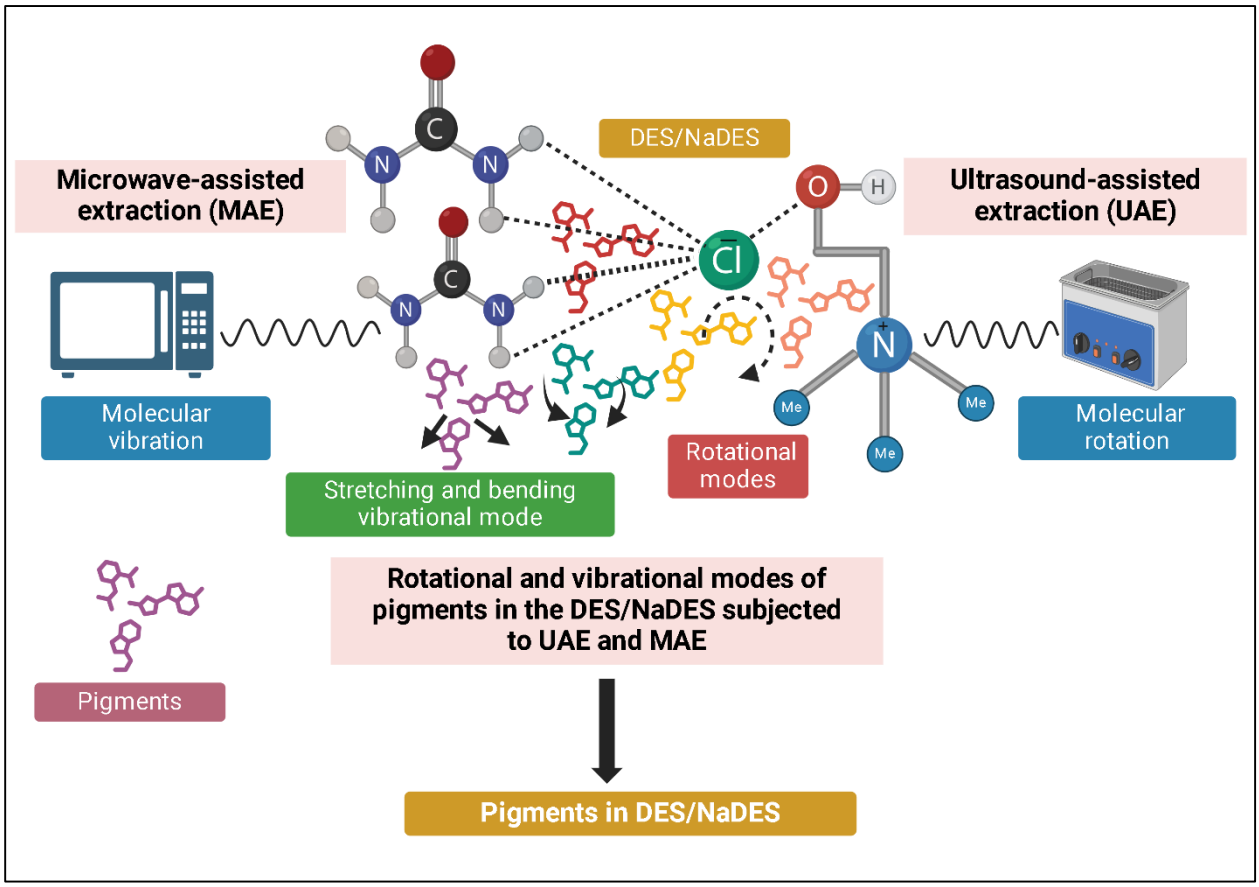

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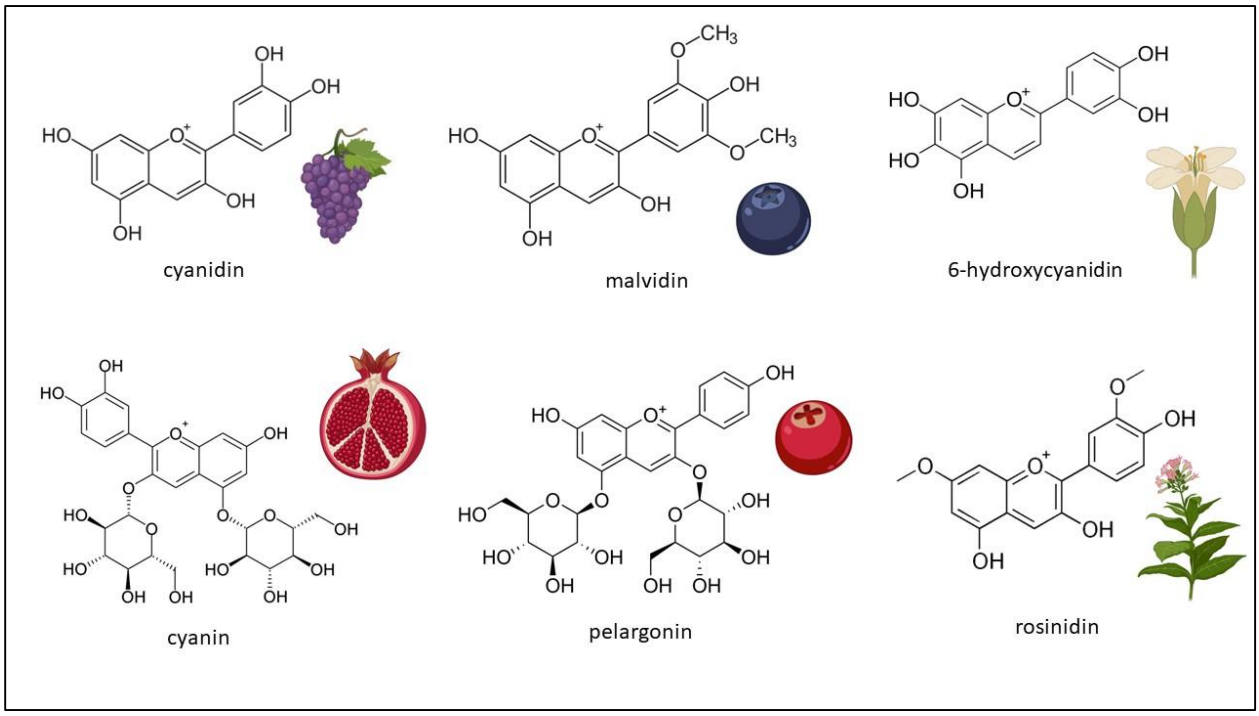

Figure 3: Chemical structure of selected anthocyanins with different functional groups and their sources cyanidin from grapes, malvidin from blueberries and bilberries, 6-hydroxycyanidin from borage, cyanin from pomegranate, pelargonin from cranberries, and rosinidin from roselle (created using BioRender.com).

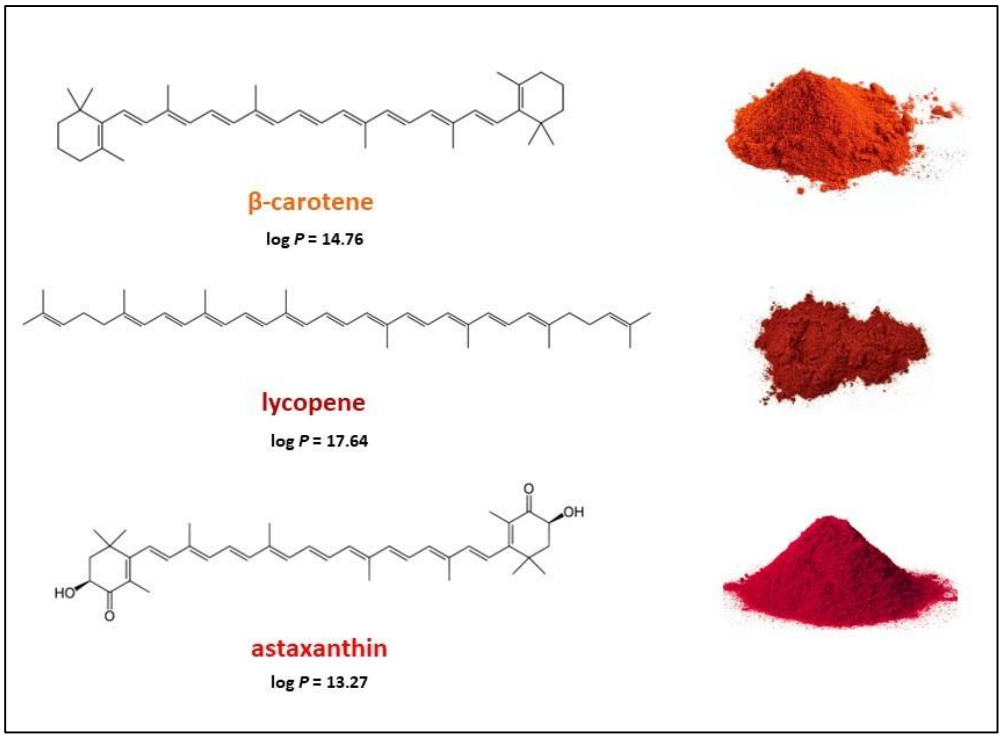

Figure 4: Chemical structure of main carotenoids with the corresponding powder represending the carotenoid extract in powdered form.

List of abbreviation

DW: Dry weight

FW: Fresh weight

DS: Dry sample

DES: Deep eutectic solvents

NaDES: Natural deep eutectic solvents (NaDES)

UAE: Ultrasound-Assisted Extraction

MAE: Microwave-Assisted Extraction

CGE: cyanidin-3-glucoside equivalents,

D3G: delphinidin-3-sambubioside equivalents,

MUAE: Microwave-Ultrasound-Assisted Extraction,

PLE: Pressurized Liquid Extraction,

HSH: High-speed homogenization

CBE: Cavitation-burst extraction