



Eutectic solvent platform for the selective isolation and one-year stabilisation of astaxanthin from crustacean shell waste

Vid Urbančič^{a,1}, Nuša Urbančič^{a,1}, Žan Lavrič^a, Bárbara M.C. Vaz^b, Sónia P.M. Ventura^b, Blaž Likozar^a, Filipa A. Vicente^{a,*}

^a National Institute of Chemistry, Hajdrihova 19, Ljubljana, 1000, Slovenia

^b CICECO-Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Aveiro, 3810-193, Portugal

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ABSTRACT

Astaxanthin is a high-value natural carotenoid with strong antioxidant activity, attractive colour properties, and broad potential for use in food, cosmetics, nutraceuticals, and pharmaceutical formulations. However, its practical application is limited by its low stability and the need for sustainable and selective extraction strategies from renewable feedstocks. In this work, crustacean shell waste was explored as a circular source of astaxanthin, using (deep) eutectic solvents (DES) as alternative extraction media. A series of DES based on choline chloride or tetrabutylammonium bromide combined with different hydrogen bond donors was screened for the selective solid-liquid extraction of astaxanthin from shrimp shell waste. Tetrabutylammonium bromide-based DES outperformed choline chloride-based systems, particularly [N_{4,4,4,4}]Br:hexanol and [N_{4,4,4,4}]Br:octanoic acid. Response surface methodology was then used to optimise the solid-liquid ratio (SLR) and extraction time. [N_{4,4,4,4}]Br:hexanol achieved an astaxanthin mass balance of ~80% with minimal co-extraction of proteins (~20%) and no extraction of minerals or chitin, with a SLR of 0.4 g mL⁻¹ and 10 min of extraction time. The long-term stability of astaxanthin in the selected DES was also evaluated for one year under different temperature and light conditions. Astaxanthin showed remarkable stability in [N_{4,4,4,4}]Br:hexanol, retaining approximately 44% of its initial content after 365 days at 25 °C in the dark while maintaining a visible orange-red colour. Kinetic modelling further confirmed this stability, estimating a half-life (t_{1/2}) of 265 and 282 days based on first- and second-order kinetic models, respectively. These results demonstrate that [N_{4,4,4,4}]Br:hexanol can act simultaneously as an efficient extraction solvent and a stabilising medium, reducing downstream processing while enabling the sustainable valorisation of crustacean shell waste into stable natural colourants.

1. Introduction

The transition from petroleum-based chemistry to renewable and circular processes is essential to address current environmental challenges, including climate change, resource depletion, and the increasing generation of waste. In this context, materials traditionally regarded as low-value residues should be reconsidered as renewable feedstocks to produce high-value compounds. This approach is central to the development of green biorefinery strategies and directly supports the principles of a circular bioeconomy, where waste generation is reduced and valuable molecules are recovered and reintegrated into productive chains [1,2]. Such strategies are also aligned with the United Nations 2030 Agenda and its 17 Sustainable Development Goals [3], as well as

with several European sustainability policies, including the European Green Deal, the Circular Economy Action Plan, the European Bioeconomy Strategy, the Zero Pollution Action Plan, and the Marine Strategy Framework Directive.

Food and agricultural residues represent one of the largest renewable waste streams worldwide. Among them, crustacean shell waste is particularly relevant, with approximately 7–9.7 million tonnes generated annually. Although this biomass is still largely underexploited, it is a rich source of valuable compounds, including chitin, proteins, minerals, lipids, and carotenoids [4]. One of the most attractive carotenoids present in crustacean shells is astaxanthin, a xanthophyll structurally related to β-carotene, lutein, and zeaxanthin [5,6]. The presence of hydroxyl and keto groups in its terminal ionone rings gives astaxanthin

* Corresponding author.

E-mail address: filipa.andre.vicente@ki.si (F.A. Vicente).

¹ Authors contributed equally.

enhanced antioxidant capacity [5], together with reported anti-inflammatory, antidiabetic, and anticancer properties [6–8]. In addition, its intense orange-red colour makes it a promising natural pigment with dual functionality, combining colouring properties with potential health-related benefits.

Astaxanthin has attracted increasing interest for applications in aquaculture, food, cosmetics, nutraceuticals, and pharmaceuticals [6]. This growing demand is reflected in the expansion of the global astaxanthin market, which was valued at US\$1.97 billion in 2025 and is expected to reach US\$6.92 billion by 2034 [9]. However, despite its high commercial and functional value, the wider application of natural astaxanthin remains limited by its sensitivity to environmental conditions. Like many natural colourants, astaxanthin is prone to degradation when exposed to light, temperature, oxygen, and different solvent environments [10]. Therefore, stabilisation strategies are often required to preserve its colour and bioactivity, including encapsulation, emulsification, protein binding, cyclodextrin complexation, and co-pigmentation. Co-pigmentation is particularly relevant for natural colourants, as it relies on non-covalent interactions between pigments and co-pigments, such as π - π stacking, hydrogen bonding, and hydrophobic interactions, which can improve colour expression and resistance to degradation [11].

The search for natural colourants has intensified in recent years, especially for food, beverages, cosmetics, and textile applications. This trend is mainly driven by consumer preference for natural ingredients, which are often perceived as safer and more sustainable than synthetic alternatives [12–15]. Nevertheless, replacing synthetic colourants with natural ones remains challenging because only a limited number of natural pigments are commercially available, and many of them suffer from poor stability, weak chromatic performance, or limited compatibility with industrial formulations [12–15]. These limitations are particularly relevant for warm colours, especially red tones, which are among the most widely used in the food industry [14–16]. In this context, astaxanthin is an attractive option because it combines a strong orange-red colour with recognised biological activity. Moreover, astaxanthin has already been approved by both the European Food Safety Authority and the Food and Drug Administration as a food colourant, with the E-code E161j [12].

Natural sources of astaxanthin include microalgae, yeast, bacteria, salmon, trout, krill, shrimp, crabs, and crayfish, as these organisms accumulate the pigment along the food chain [6–8]. Among these, microalgae are currently one of the most explored sources due to their high astaxanthin accumulation under stress conditions [5,7,8]. However, microalgal production presents important limitations, since pigment accumulation is difficult to control and stress conditions often induce the formation of thick and rigid cell walls, which hinder astaxanthin extraction [17]. In contrast, crustacean shell waste offers an abundant, inexpensive, and underused alternative source. Its valorisation is particularly attractive because it combines the recovery of a high-value natural pigment with the reduction of seafood-processing waste, fitting well within biorefinery and circular economy concepts.

Conventional astaxanthin extraction is commonly performed using organic solvents, acids, edible oils, enzymatic methods, or intensification techniques such as microwave-assisted extraction and ultrasound-assisted extraction [18,19]. Although these approaches can be effective, they may involve high solvent consumption, limited selectivity, additional energy input, complex downstream processing, or poor compatibility with industrial-scale implementation. Therefore, there is a clear need for alternative extraction platforms that are efficient, selective, cost-effective, and more sustainable.

Ionic liquids [20] and (deep) eutectic solvents (DES) have been explored as alternative solvents for the extraction of bioactive compounds, including astaxanthin [18,19,21,22]. Among these, DES have attracted particular attention due to their simple preparation, tuneable properties, and promising performance in organic catalysis, biotransformations, and molecular extractions [23]. Recent studies have

further demonstrated the versatility of DES for the recovery of different classes of natural bioactive compounds from biomass [24]. DES are typically prepared by combining a hydrogen bond acceptor and a hydrogen bond donor, resulting in liquids whose polarity, hydrophobicity, viscosity, and hydrogen-bonding capacity can be tailored according to the target application [23,25]. Although DES are often compared with ILs due to their designer-solvent character, they generally offer several advantages, including easier and cheaper preparation, fewer disposal concerns, and often lower toxicity profiles [23]. In the case of astaxanthin, DES are especially promising because they may act not only as extraction solvents but also as stabilising media. Their structured hydrogen-bond networks may interact with the hydroxyl and keto groups of astaxanthin, while hydrophobic domains may favour solubilisation of the polyene chain and reduce exposure to oxidative degradation. This dual function could provide a co-pigmentation-like stabilisation effect, improving pigment preservation without requiring additional stabilising agents.

In this study, a DES-based platform was developed for the selective extraction and long-term stabilisation of astaxanthin from shrimp shell waste. Various DES based on choline chloride and tetrabutylammonium bromide were screened in combination with hydrogen bond donors of different polarity and hydrophobicity. The most promising systems were then optimised using response surface methodology, with solid-liquid ratio and extraction time as the key parameters. The long-term stability of astaxanthin in the selected DES was evaluated for up to one year under different temperature and light conditions. Additionally, kinetic modelling was applied to describe astaxanthin degradation under the various storage conditions and to determine the corresponding degradation rates and half-life values. This study demonstrates that appropriately designed DES can simultaneously enable the selective isolation and stabilisation of astaxanthin, providing a sustainable route for the valorisation of crustacean shell waste into a stable natural colourant.

2. Materials and methods

2.1. Materials

Acetic acid (glacial) was obtained from Merck. Absolute anhydrous ethanol (99.9% purity) was sourced from Carlo Erba. Astaxanthin, choline chloride (98% purity), lactic acid (85% purity), malonic acid (99% purity), octanoic acid (99% purity), hexanol (99% purity), lauric acid (98% purity), and citric acid monohydrate were purchased from Sigma Aldrich. Glycerol (98% purity) was obtained from Fluorochem. Tetrabutylammonium bromide ($[N_{4,4,4,4}]Br$, $\geq 97\%$ purity) and ethylene glycol were purchased from Fluka Chemie AG. Butanol (100%) and disodium phosphate were acquired at Kemika and Alfa Aesar, respectively. Argentine red shrimp shells were kindly provided by a local fish market in Ljubljana, Slovenia.

2.2. Methods

2.2.1. DES preparation

DES preparation was carried out by mixing each hydrogen bond acceptor (HBA) with several hydrogen bond donors (HBDs) in a molar ratio of 1:2, respectively, at 60–80 °C until a clear liquid mixture was obtained. HBAs and HBDs chemical structures are presented in Fig. 1.

2.2.2. Solid-liquid extraction

Shrimp shells were first washed three times with tap water, then three times with distilled water, and stored at -20 °C until further use. Before extraction, the shells were ground in a mortar while frozen with liquid nitrogen. For the conventional system, acetic acid was used, and various DES were tested as alternative systems. The solid-liquid ratio (SLR) is expressed throughout as the mass of frozen shrimp shells per volume of solvent (g mL^{-1}). Initial solvent screening was performed at an SLR of 0.2 g mL^{-1} (typically 2 g of frozen shells in 10 mL of solvent)

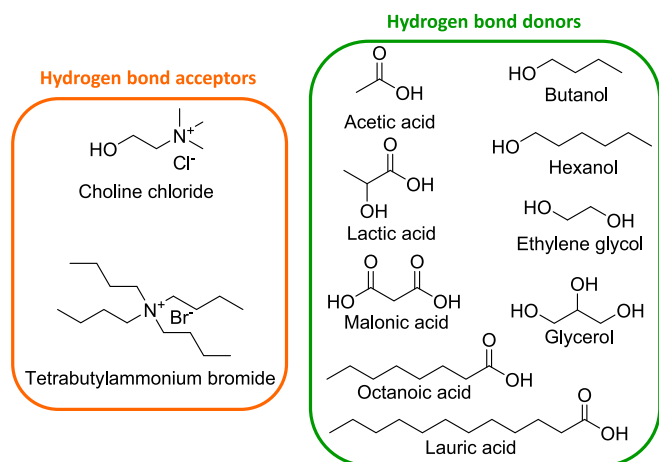


Fig. 1. HBAs and HBDs structure of the different DES studied in this work.

for 1 h at 25 °C and 280 rpm in small reactors using a Radleys Carousel™ apparatus. The performance of each system was evaluated by UV–Vis spectroscopy using a Synergy H1 Hybrid Multi-Mode Reader (BioTek). Absorbance at 482 nm was used to quantify total astaxanthin, including free and esterified forms without distinguishing them individually, while absorbance at 280 nm was used to estimate total protein concentration. The absorbance of the corresponding solvent blank was subtracted from each measurement. The calibration curves for astaxanthin and total proteins were prepared using free astaxanthin and bovine serum albumin standards, respectively, are presented in Fig. S1 of the Supporting Information (SI). Once the best DES had been selected, a response surface methodology was used to simultaneously optimise different parameters. A 2² factorial design was performed to evaluate the influence of the SLR and extraction time (independent variables) on the solvent's ability to extract astaxanthin (dependent variable - $c_{\text{astaxanthin}}$). A similar study was performed for total proteins to evaluate the selectivity of the system. Detailed data are provided in Table S1 of SI for the coded and decoded matrices. The results obtained were statistically analysed at a 95% confidence level. The software Statsoft Statistica 10.0© was used for statistical analysis and preparation of the response surface plots.

Mass balance (%) was determined for the best systems in comparison with the conventional system, namely acetic acid, according to Eq. (1) (Eq. (1)):

$$\text{Mass Balance (\%)} = \frac{C_{\text{astaxanthin after extraction}}}{C_{\text{astaxanthin initial}}} \times 100 \quad (1)$$

where $C_{\text{astaxanthin after extraction}}$ and $C_{\text{astaxanthin initial}}$ correspond to astaxanthin concentration after the extraction with each system and the initial colourant concentration in the biomass, respectively.

2.2.3. Kinetic modelling of astaxanthin decomposition

The decomposition rate of astaxanthin at different storage temperatures (25, 40 and 60 °C) in dark conditions was computed by the modified Arrhenius rate equation (Eq. (2)):

$$k = k_{\text{ref}} \cdot e^{-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right)} \quad (2)$$

where k_{ref} is the reaction rate constant, calculated at the reference temperature.

The reaction rate equation was studied for a 1st and 2nd order kinetics. The reaction rate equations (Eqs. (3) and (4)) correspond to each order above, respectively:

$$r_{\text{decomposition}} = k \cdot C_{\text{ast}}; k \text{ in units } [\text{day}^{-1}] \quad (3)$$

$$r_{\text{decomposition}} = k \cdot C_{\text{ast}}^2; k \text{ in units } [\text{L mol}^{-1} \text{ day}^{-1}] \quad (4)$$

where k is the reaction rate constant, calculated by Eq. (2), while C_{ast} is the molar concentration of astaxanthin in mol L⁻¹.

The mass balance equation for astaxanthin was calculated with Eq. (5):

$$\frac{dC_{\text{ast}}}{dt} = -r_{\text{decomposition}} \quad (5)$$

The kinetic parameters were estimated by simultaneous nonlinear fitting of the three independent experimental datasets. For each kinetic model (1st and 2nd order), the model parameters were determined by minimizing the overall sum of squared residuals ($SS_{\text{res, total}}$) across all experimental points, calculated according to Eq. (6):

$$SS_{\text{res, total}} = \sum_{j=1}^{N_{\text{exp}}} \sum_{i=1}^{n_j} (C_{\text{exp, ij}} - C_{\text{model, ij}})^2 \quad (6)$$

where N_{exp} is the number of independent experimental datasets, n_j is the number of experimental data points in dataset j , $C_{\text{exp, ij}}$ is the experimentally determined astaxanthin concentration, and $C_{\text{model, ij}}$ is the corresponding model-predicted concentration. Thus, the kinetic parameters were obtained from a global fit in which all three independent datasets were fitted simultaneously using the same model parameters.

The numerical regression analysis was performed using Matlab 2025b software. The concentration profiles were solved as a function of time with the ODE15s solver, while the regression analysis was performed with *fminsearch* function implementing the Nelder-Mead algorithm. Parameter estimation was performed using the *lsqnonlin* function with the Levenberg-Marquardt algorithm, which enables the determination of confidence intervals.

The goodness of fit was evaluated separately for each experimental dataset using the coefficient of determination (R^2). For each dataset j , R^2 was calculated according to Eqs. (7)–(9):

$$R_j^2 = 1 - \frac{SS_{\text{res, j}}}{SS_{\text{tot, j}}} \quad (7)$$

$$SS_{\text{res, j}} = \sum_{i=1}^{n_j} (C_{\text{exp, ij}} - C_{\text{model, ij}})^2 \quad (8)$$

$$SS_{\text{tot, j}} = \sum_{i=1}^{n_j} (C_{\text{exp, ij}} - \bar{C}_{\text{exp, ij}})^2 \quad (9)$$

where $\bar{C}_{\text{exp, ij}}$ represents the mean experimental astaxanthin concentration of dataset j . The R^2 values were, therefore, calculated individually for each dataset to assess the goodness of fit of the corresponding kinetic model.

3. Result and discussion

3.1. Screening of different DES

As task-specific solvents, DES can be designed according to the intended application. In this case, the aim is the selective extraction of astaxanthin, a hydrophobic compound, from shrimp shell waste. For this reason, tetrabutylammonium bromide ([N_{4,4,4,4}]Br) was selected as the most hydrophobic HBA and compared to choline chloride ([Ch]Cl), which is one of the HBAs most used. Regarding the HBDs, ethylene glycol, glycerol, acetic acid, lactic acid, and malonic acid were selected as common alcohols and organic acids, respectively, and compared with butanol, hexanol, octanoic acid, and lauric acid, which have longer alkyl side chains, thus conferring greater hydrophobicity to the DES. Additional information on the DES components, including molar mass, CAS number, and experimental water content, is provided in Table S2 of SI,

together with information on DES formation and their physical state at 25 °C. This table indicates that it was not possible to form [Ch]Cl-based DES with the more hydrophobic HBDs, possibly due to an insufficient number of groups involved in hydrogen bond formation. The DES liquid at room temperature were screened to evaluate their ability to disrupt the cells of shrimp shells and enable the extraction of astaxanthin. As proteins are present in considerably higher amounts in the biomass, they were also analysed and considered as the main contaminant (results in Fig. 2). Here, acetic acid was used as the conventional solvent for astaxanthin extraction, serving as a control. Regarding the use of alternative solvents, [Ch]Cl-based DES showed a poor ability to disrupt the shrimp shell cells and/or affinity towards the colourant, resulting in the extraction of low amounts of astaxanthin and proteins. In contrast, [N_{4,4,4,4}]Br-based DES showed very promising results for the extraction of the natural colourant, particularly [N_{4,4,4,4}]Br:butanol and [N_{4,4,4,4}]Br:hexanol, which produced results comparable to the conventional system, namely acetic acid. The higher selectivity observed for these DES compared with the [Ch]Cl-based systems may be related to their more hydrophobic character, which provides a favourable environment for the extended non-polar region of astaxanthin while resulting in lower affinity for several components of the complex chitin-protein-mineral matrix. Nevertheless, the contribution of individual solvent-biomass interactions to this selectivity remains difficult to isolate. Moreover, these DES were significantly more selective for the extraction of astaxanthin than acetic acid, reducing protein contamination by at least four-fold, from $16.5 \pm 0.8 \text{ mg mL}^{-1}$ with acetic acid to $3.7 \pm 0.2 \text{ mg mL}^{-1}$ and $2.83 \pm 0.05 \text{ mg mL}^{-1}$ with [N_{4,4,4,4}]Br:butanol and [N_{4,4,4,4}]Br:hexanol, respectively. Therefore, these DES were selected as the most promising for the natural colourant extraction. [N_{4,4,4,4}]Br:octanoic acid was also chosen for the next optimisation step, as it showed the lowest protein contamination ($1.7 \pm 0.3 \text{ mg mL}^{-1}$).

3.2. Optimization of the SLR and extraction time

Factorial planning is a useful tool for optimising multiple conditions simultaneously, so it was used to study the influence of SLR and

extraction time on the extraction of astaxanthin and total proteins. These results are shown in Figs. 3–5 for [N_{4,4,4,4}]Br:butanol, [N_{4,4,4,4}]Br:hexanol, and [N_{4,4,4,4}]Br:octanoic acid, respectively, with red regions indicating higher concentrations and green regions lower concentrations of each compound. Tables S3–S8 present the experimental and theoretical results. The fitted responses agreed with the experimental values, with R^2 values of 0.913, 0.973 and 0.951 for astaxanthin and 0.987, 0.954 and 0.819 for total proteins using [N_{4,4,4,4}]Br:butanol, [N_{4,4,4,4}]Br:hexanol and [N_{4,4,4,4}]Br:octanoic acid, respectively. The regression coefficients, standard deviation, t-student, and p -values were also calculated and are reported in Tables S9–S14.

Considering the aim of this work, namely the selective extraction of astaxanthin from shrimp shell waste, the best conditions are represented by a red region for astaxanthin and a green region for total proteins. In this context, this goal may be difficult to achieve with [N_{4,4,4,4}]Br:butanol as the extraction solvent (Fig. 3), since the conditions in the red region for astaxanthin mostly overlap with those for total proteins. To extract the highest possible concentration of the colourant, a SLR higher than 0.30 g mL^{-1} should be selected, while performing the extraction for a maximum of 20 min. Time may lead to some differences in the amount of compounds extracted, though it is more relevant for total proteins, as these results present a mirror plane around 50 min of extraction. This indicates that 30 or 70 min of extraction time should yield approximately the same performance. This is further corroborated by the Pareto charts shown in Fig. S2 of the SI, which indicate that the SLR has the most statistically significant effect on the extraction of the compounds.

In contrast, the response surface and contour plots for [N_{4,4,4,4}]Br:hexanol and [N_{4,4,4,4}]Br:octanoic acid show very interesting and promising results, as the maximum estimation of astaxanthin concentration exceeds $40 \mu\text{g mL}^{-1}$, while the total protein concentration is only around 6 mg mL^{-1} . This total protein concentration represents a four-fold reduction in the maximum amount of protein estimated during the acetic acid-based extraction, indicating a considerable improvement in the selectivity of the overall process. Fig. 4 presents the optimisation results for the [N_{4,4,4,4}]Br:hexanol-based system, showing a monotonic extraction of both compounds over time. Conversely, increasing the SLR

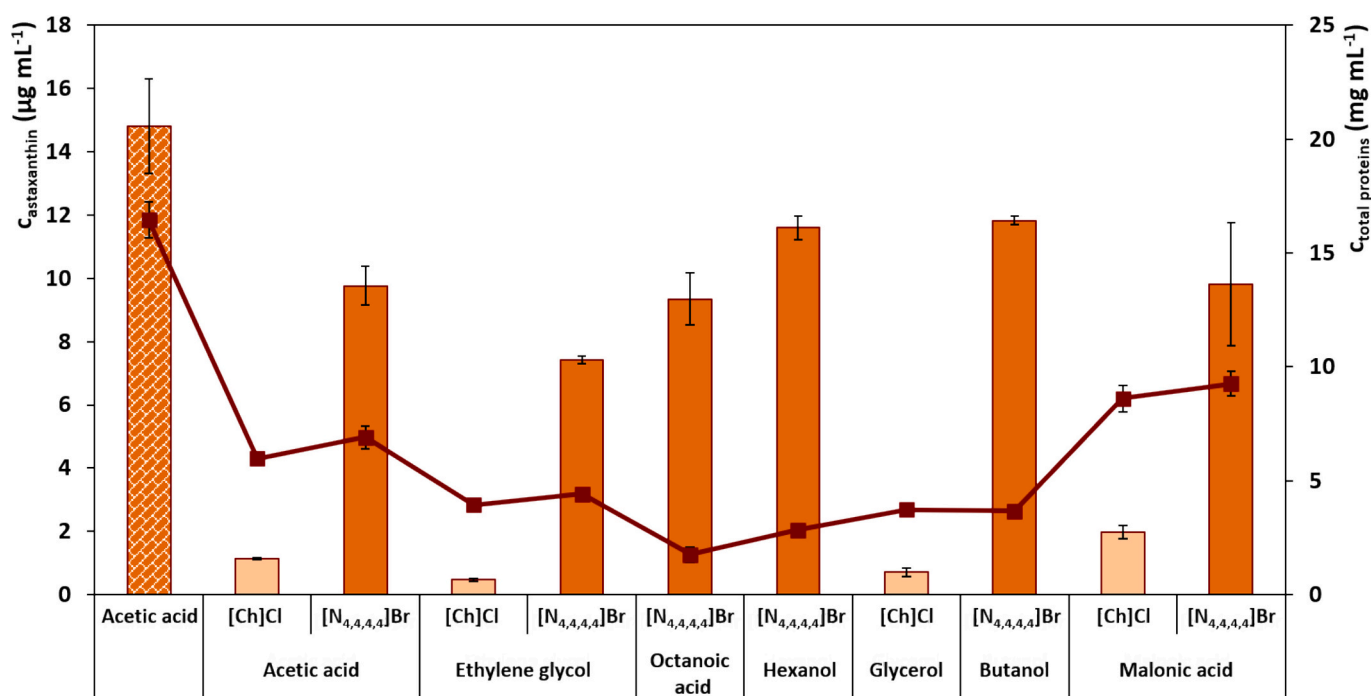


Fig. 2. Astaxanthin (bars) and total protein (line) concentrations after SLE with acetic acid (conventional solvent) and the different DES. HBDs are indicated below each group, while the corresponding HBA ([Ch]Cl or [N_{4,4,4,4}]Br) is indicated below each bar. Error bars represent the standard deviation of three independent extractions ($n = 3$).

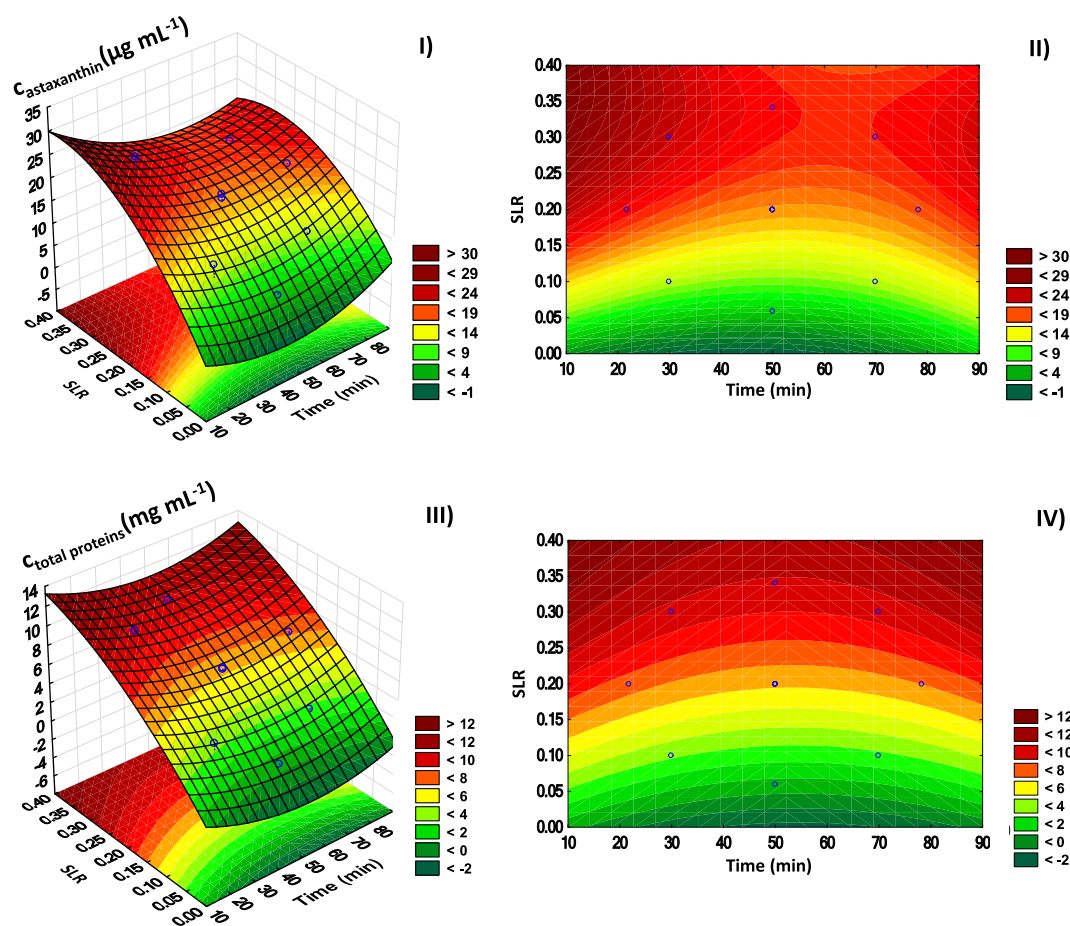


Fig. 3. Response surface plots (left) and contour plots (right) of the concentration of astaxanthin (I and II) and total proteins (III and IV) after the solid-liquid extraction with $[N_{4,4,4,4}]\text{Br}:\text{butanol}$ (1:2 molar ratio).

further enhances the extractive performance for each compound. This once again indicates that the SLR is the independent variable with the most statistically significant effect on compound extraction, as validated by the Pareto charts shown in Fig. S3 of the SI. Therefore, the most promising extraction conditions are a SLR greater than 0.25 g mL^{-1} and an extraction time of less than 20 min to save energy and time.

Considering $[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$, most experimental conditions result in the extraction of very low amounts of total proteins. However, to achieve a successful extraction of the colourant, the SLR should be higher than 0.30 g mL^{-1} and the extraction period should be longer. According to Fig. S4 on SI, within these two parameters, only SLR has a statistically significant influence on astaxanthin extraction at a 95% confidence level, as demonstrated by the Pareto charts. This means that, although extraction time appears to affect the extractive performance, this parameter is not particularly relevant when selecting the best extraction conditions. Time is only a significant parameter for increasing the extraction of total proteins, which is undesirable in this case. Therefore, keeping the extraction time under 20 min for both $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ and $[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$, and a SLR above 0.30 g mL^{-1} , seems to be the best option.

3.3. Comparison of astaxanthin and total proteins concentration in optimal conditions

Following the optimisation study, new extractions were performed at an SLR of 0.4 g mL^{-1} and an extraction time of 10 min, selected as a practical condition that could reduce operating time and associated costs. The two most promising DES, $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ and $[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$, were tested alongside acetic acid as the control. An SLR of

0.2 g mL^{-1} was also tested, as this has been reported as the optimal condition for extraction with acetic acid [26]. The results are presented in Table 1 (See Table 2.), with astaxanthin concentrations expressed in $\mu\text{g mL}^{-1}$ and $\mu\text{g g}^{-1}$ of shells, and total protein concentrations in mg mL^{-1} and mg g^{-1} of shells. The mass balance was also determined by considering the amount of each compound initially present in the biomass. As aforementioned, for the selective extraction of astaxanthin from the biomass, the aim is to extract the highest amount of the colourant, while extracting the lowest amount of total proteins. For this reason, the best system should be represented by the highest mass balance for astaxanthin while also displaying the lowest mass balance for total proteins. This is the case for $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ with a SLR of 0.40 g mL^{-1} , where more than 80% of astaxanthin can be recovered ($80 \pm 3 \mu\text{g g}_{\text{shells}}^{-1}$) while extracting only $\sim 20\%$ of total proteins ($14.3 \pm 0.7 \text{ mg g}_{\text{shells}}^{-1}$). It is possible that the co-extracted proteins are cuticular structural proteins and/or carotenoproteins, such as crustacyanins, to which astaxanthin is non-covalently bound, although to a lesser extent in shrimps than in other crustaceans such as lobsters or crabs [10]. This may explain why complete selectivity was not achieved. It should also be noted that the pellet retained a slight orange colour after extraction, indicating that at least part of the astaxanthin not recovered in the extract remained in the shrimp shell residue. Moreover, no significant degradation is expected during the 10 min extraction at 25°C , given the stability of astaxanthin in the DES under these conditions, as demonstrated by the subsequent stability study.

From a practical perspective, the use of an SLR of 0.4 g mL^{-1} is also advantageous since twice the amount of biomass can be processed with the same volume of solvent compared with an SLR of 0.2 g mL^{-1} . This corresponds to a 50% reduction in the amount of solvent required *per*

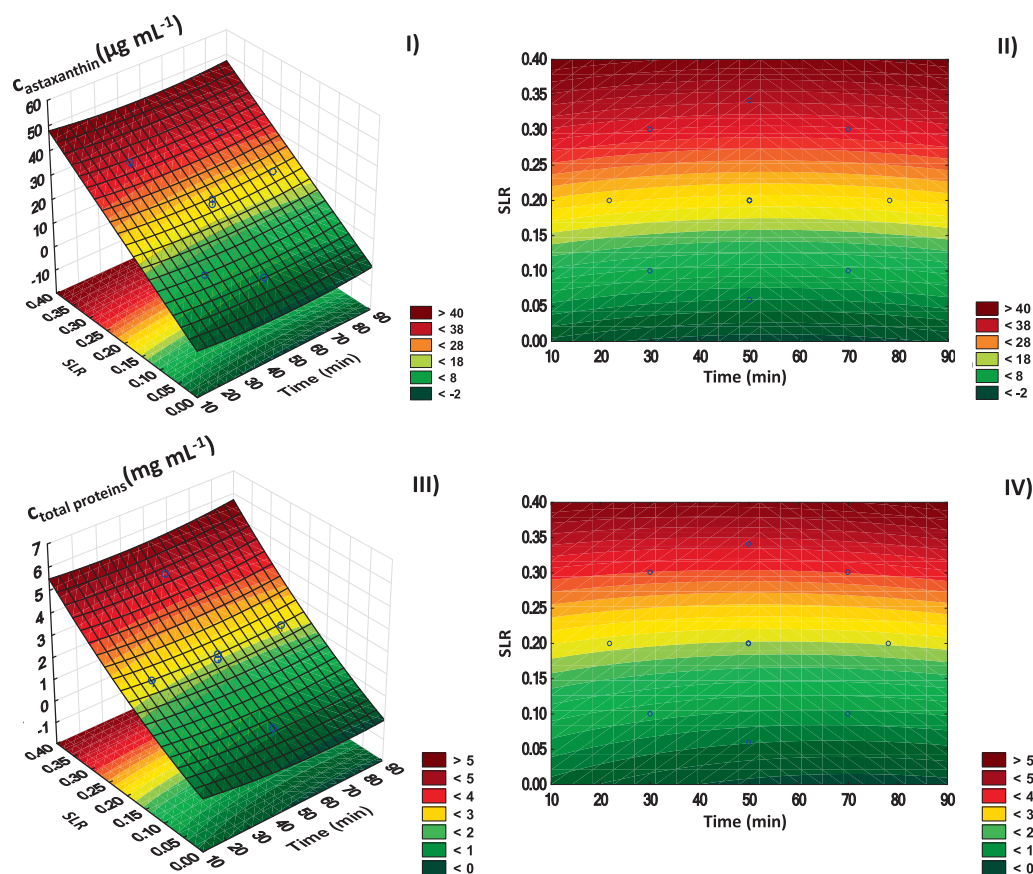


Fig. 4. Response surface plots (left) and contour plots (right) of the concentration of astaxanthin (I and II) and total proteins (III and IV) after extraction with $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ (1:2 molar ratio).

mass of biomass, which may reduce solvent consumption and associated costs and be advantageous for future scale-up. Furthermore, for $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$, increasing the SLR maintained the astaxanthin mass balance while reducing protein co-extraction from $30 \pm 1\%$ to $23 \pm 1\%$. The lower protein co-extraction, together with the lower amount of solvent required *per* mass of biomass, may also facilitate further downstream processing. Nevertheless, further scale-up studies, including solvent recovery and recycling, will be required to fully assess the economic feasibility of the process.

In conclusion, these results outperform several literature reports, in which astaxanthin extraction yields of up to $\sim 59 \mu\text{g g}_{\text{shells}}^{-1}$ were obtained using choline chloride:lactic acid-based DES with ethanol as a co-solvent ($\leq 45\%$) combined with extraction assisted by ultrasound (UAE) [27], microwave irradiation (MAE) [18], or conventional solid-liquid extraction [22]. The use of lysine: citric acid-based DES has also been reported to successfully extract $112.8 \mu\text{g g}_{\text{shells}}^{-1}$ of astaxanthin, although this was achieved after the pre-treatment of the biomass with hydrochloric acid to remove minerals and with UAE at higher temperatures (40°C) [19]. To date, only one study has reported the solid-liquid extraction of astaxanthin from crustacean shell waste using DES at 25°C with higher extraction efficiency than the present work [28]. In that study, multiple combinations of menthol and carboxylic acids (C2–C10) at a 1:1 molar ratio were evaluated using mechanical stirring for 20 min, resulting in astaxanthin yields ranging from 180 to $203 \mu\text{g g}_{\text{shells}}^{-1}$. However, the study focused exclusively on astaxanthin recovery and did not assess the purity of the extracts or the co-extraction of other shell components. Indeed, to the best of our knowledge, the available studies on astaxanthin extraction from crustacean shell waste using DES have focused mainly on astaxanthin recovery and do not report quantitative protein co-extraction or other selectivity metrics that can be

directly compared with the present results. Therefore, while extraction yields can be compared, a quantitative comparison of extraction selectivity is currently not possible. In contrast, the present work demonstrates the selective astaxanthin extraction under mild conditions (25°C , 10 min, and magnetic stirring), with $\sim 20\%$ of the protein initially present in the shrimp shells co-extracted and no detectable extraction of minerals or chitin. Further discussion of the extraction selectivity is provided in SI (see Table S15 and Figs. S5–S6). Moreover, it is important to note that only studies involving the extraction of astaxanthin from crustacean shell waste were compared, due to differences in biomass complexity and pigment location. Even among crustacean shell wastes, differences in species, initial astaxanthin content and shell composition may influence the extraction performance and should be considered when comparing extraction yields between studies. In crustacean shells, astaxanthin is mainly bound to proteins (e.g., crustacyanin) and embedded within the chitin-protein matrix, often in its esterified form. These interactions, together with the mineralised structure, hinder its isolation. In contrast, in microalgae and yeast, this colourant is predominantly accumulated in cytoplasmic lipid droplets, mostly as fatty acid esters, whereas in bacteria, it is primarily associated with the cytoplasmic membrane [8].

3.4. Long-term storage stability of astaxanthin under different conditions

Natural colourants are highly sensitive to environmental conditions, particularly temperature, light, and solvents, as well as to auto-oxidation processes, which can significantly limit their practical application [10]. Evaluating their thermal and light stability in the presence of multiple solvents is therefore a priority, especially for applications where the colourant is in solution. In this context, the long-term stability of

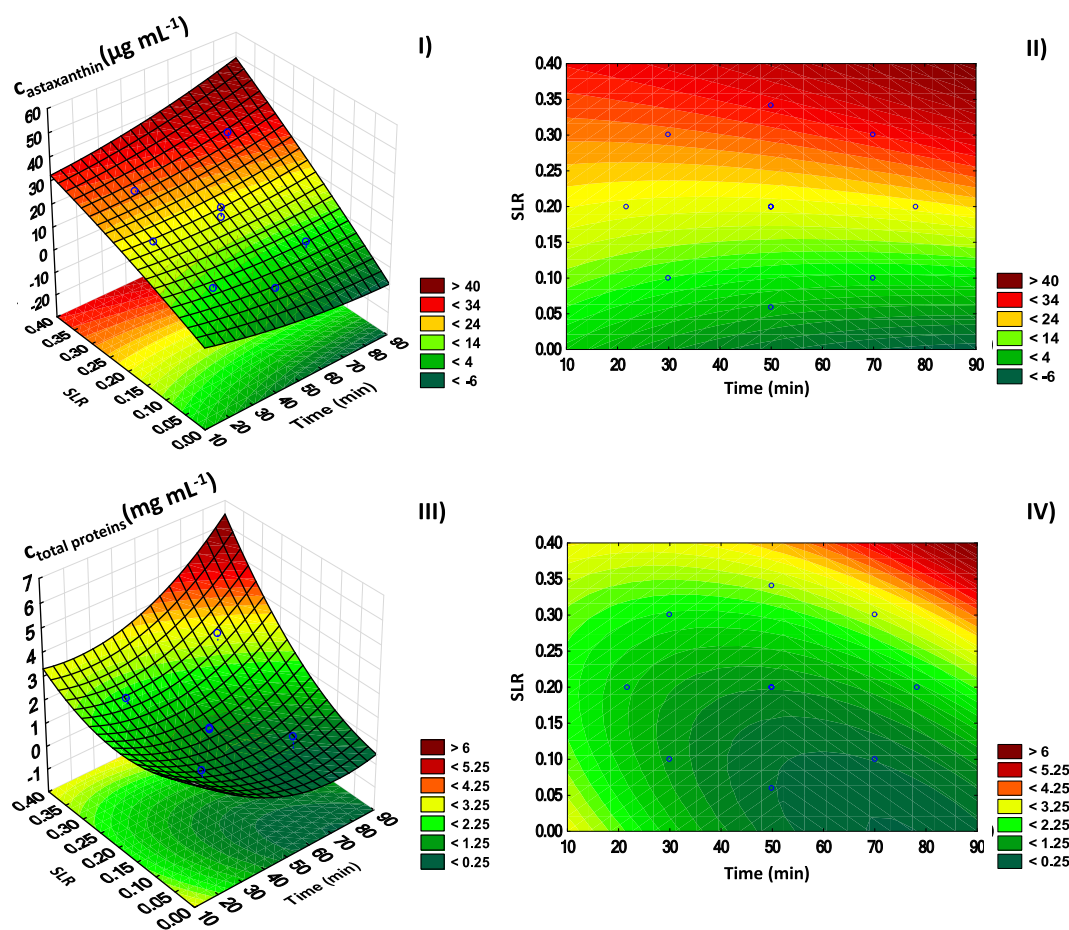


Fig. 5. Response surface plots (left) and contour plots (right) of the concentration of astaxanthin (I and II) and total proteins (III and IV) after extraction with $[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$ (1:2 molar ratio).

Table 1

Extractive performance of acetic acid, $[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$ and $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ for astaxanthin and total proteins, under the optimal conditions (SLR = 0.2 and 0.4 g mL⁻¹, and extraction time = 10 min).

Solvent	SLR	Astaxanthin			Total proteins		
		C _{astaxanthin} (μg mL ⁻¹)	C _{astaxanthin} (μg g _{shells} ⁻¹)	Mass balance (%)	C _{total proteins} (mg mL ⁻¹)	C _{total proteins} (mg g _{shells} ⁻¹)	Mass balance (%)
Acetic acid	0.2	13.7 ± 0.4	68 ± 2	69 ± 2	14.2 ± 0.4	71 ± 2	100 ± 3
	0.4	25 ± 1	63 ± 2	64 ± 3	29.8 ± 0.7	74 ± 2	100 ± 4
$[N_{4,4,4,4}]\text{Br}:\text{octanoic acid}$	0.2	13.6 ± 0.2	68 ± 1	69 ± 1	3.5 ± 0.3	17 ± 2	28 ± 3
	0.4	24.1 ± 0.9	60 ± 2	61 ± 3	4.4 ± 0.6	11 ± 2	18 ± 3
$[N_{4,4,4,4}]\text{Br}:\text{hexanol}$	0.2	15.5 ± 0.7	77 ± 4	79 ± 5	3.7 ± 0.1	18.8 ± 0.6	30 ± 1
	0.4	32 ± 1	80 ± 3	82 ± 4	5.7 ± 0.3	14.3 ± 0.7	23 ± 1

Table 2

Comparative astaxanthin degradation, i.e. loss (%), in $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ and control (acetic acid) systems after 365 days of storage under different temperature and light conditions.

Conditions	Total loss (%)												
	Day 15	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	Day 210	Day 240	Day 270	Day 300	Day 330	Day 365
25 °C dark	19.8	29.5	29.4	35.4	41.5	42.6	44.4	44.5	48.7	49.4	53.6	53.9	56.3
25 °C light	22.6	32.5	38.2	47.4	52.6	54.5	57.7	59.4	64.9	66.8	74.2	78.2	81.2
40 °C dark	23.9	32.9	38.8	48.8	59.0	61.2	64.8	69.7	73.6	74.6	79.8	80.2	80.9
60 °C dark	36.1	53.2	79.2	90.4	93.2	94.2	100	100	100	100	100	100	100
Control 60 °C dark	100	100	100	100	100	100	100	100	100	100	100	100	100

astaxanthin was studied at different temperatures and under different light conditions, specifically at 25, 40 and 60 °C, and in both light and dark environments at 25 °C. Figs. 6 and 7, and Tables 2, S16, and S17 (from SI) show the stability of astaxanthin and co-extracted proteins in

$[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ over 365 days of storage under these conditions. The stability of the compounds was also evaluated in acetic acid as the control; however, since this sample formed a gel [26], it was only possible to analyse it at 60 °C in the dark (results shown in Tables S16

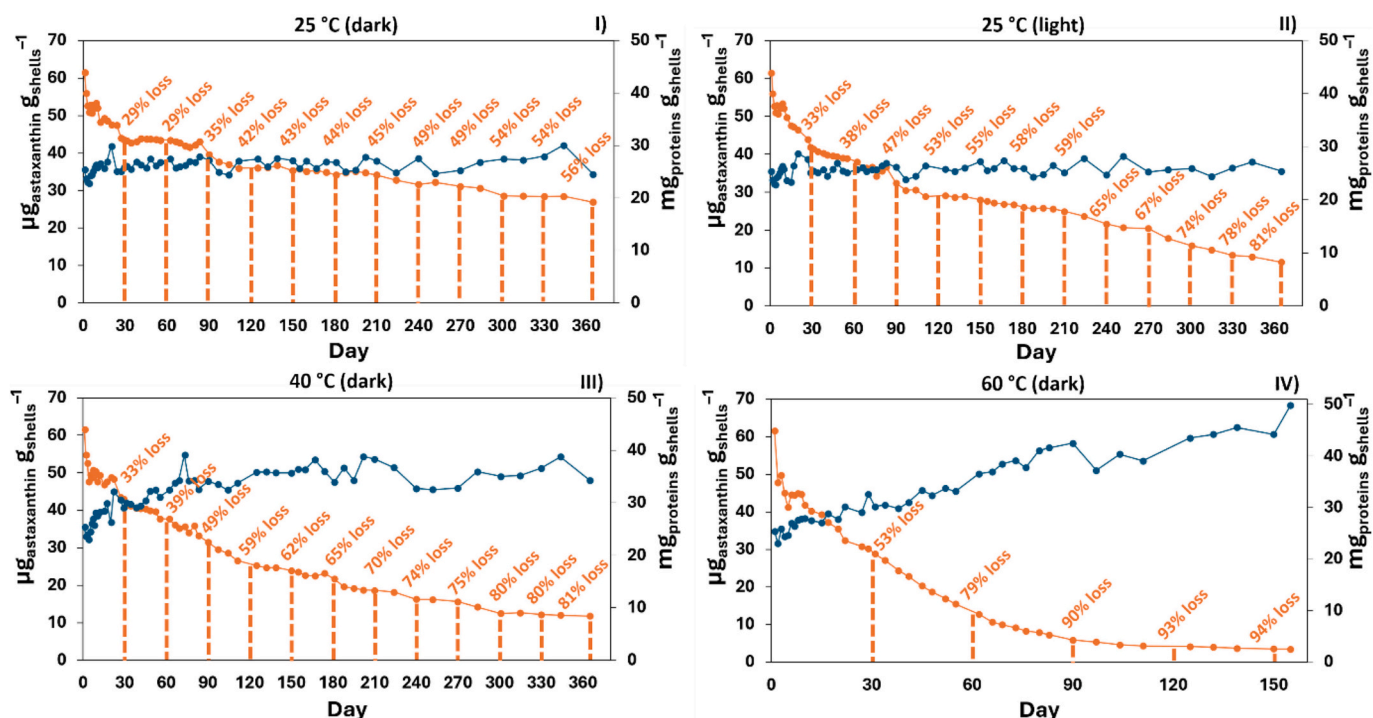


Fig. 6. Long-term stability of astaxanthin (orange) and proteins (blue) in $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ during 365-day storage under different conditions: I) 25 °C in the dark, II) 25 °C under light exposure, III) 40 °C in the dark, and IV) 60 °C in the dark. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and S17 in SI).

In the acetic acid extract, astaxanthin was completely degraded after 15 days, as indicated by the disappearance of its characteristic UV–Vis absorption band (see Fig. S7 in SI). At the same time, the apparent protein concentration increased. Since the stability study was performed on the extract after removal of the shrimp shell biomass, this increase cannot result from further protein extraction. Instead, it may reflect protein denaturation, aggregation and/or degradation, with associated changes in aromatic-residue absorbance affecting the analytical response at 280 nm and leading to an apparent increase in protein concentration. Moreover, this sample developed turbidity after 90 days (see Fig. 7) and was thus excluded from subsequent analysis. At the same temperature and in the dark, astaxanthin persisted longer in the DES: ~46.8% remained after 1 month at 60 °C, whereas its characteristic absorption band had disappeared from the acetic acid control after 15 days. Complete degradation in the DES was observed only after 5 months. At 40 °C, ~51% of astaxanthin was still present after 3 months, followed by a gradual decline, reaching ~81% loss after 12 months. At 25 °C under light exposure, approximately 50% and 81% losses were observed after 3 and 12 months, respectively. Notably, at 25 °C and in the absence of light, degradation was substantially reduced, with only approximately 50% and 56% losses after 9 and 12 months, respectively. Furthermore, Fig. 7 clearly shows that even after one year, the extracts still retain a vibrant orange colouration. Regarding the proteins present in solution, these appeared stable at 25 °C under both light conditions throughout the duration of the study. However, at higher temperatures, the apparent protein concentration increased. As discussed for the control, this cannot result from further protein extraction and may instead be associated with temperature-induced changes in the proteins that affect their analytical response at 280 nm.

These results compare favourably with previously DES-based stabilisation systems reported to date, as shown in Table 3. In literature, the best results report a half-life ($t_{1/2}$) of ~69 days for pure astaxanthin ester at 4 °C using DES-based microemulsions containing menthol:octanoic acid [29], and the retention of approximately 90% of astaxanthin after

30 days at 25 °C in the presence of light in menthol:acetic acid [30]. Interestingly, all literature studies rely on terpenoid-based DES, often combined with fatty acids. According to this stability data, temperature has a more significant effect on astaxanthin degradation than light exposure. However, in our study, temperature and light appear to have a similar influence over short term storage. An increase of 15 °C (from 25 to 40 °C) resulted in a degradation level comparable to that observed under light exposure, with approximately 50% of astaxanthin remaining after 3 months at 40 °C in the dark and after 3 months at 25 °C under light. Nonetheless, our results also showed that over longer storage periods at room temperature, light exposure clearly accelerated degradation, resulting in approximately 81% loss after 12 months, compared to approximately 50% and 56% losses after 9 and 12 months, respectively, in the dark. This long-term stability may be attributed to a co-pigmentation-like stabilising effect involving hydrophobic interactions and hydrogen bonding between the DES and astaxanthin. In classical co-pigmentation, a pigment associates with a separate co-pigment, often an aromatic molecule, through non-covalent interactions that may include π – π stacking, hydrophobic interactions and hydrogen bonding [11]. The proposed effect in the present system may involve some of the same types of interactions, but the DES functions as the solvent rather than as a separate co-pigment. Hydrogen-bonding and van der Waals interactions between DES components and carotenoids have previously been associated with improved carotenoid solubility and stability [29,31]. In the present system, the highly structured hydrogen-bond network of the DES can interact with the hydroxyl and keto groups of astaxanthin, reducing its conformational flexibility and lowering its susceptibility to oxidation. Simultaneously, the relatively hydrophobic and bulky nature of $[N_{4,4,4,4}]\text{Br}:\text{hexanol}$ appears to contribute to the formation of a microenvironment that solubilises the polyene chain while providing steric shielding, which may limit oxygen diffusion and slow radical propagation. This effect may also be reinforced by the higher viscosity of the DES, as viscosity has been suggested to contribute to the enhanced stability of pigments in DES media [32].

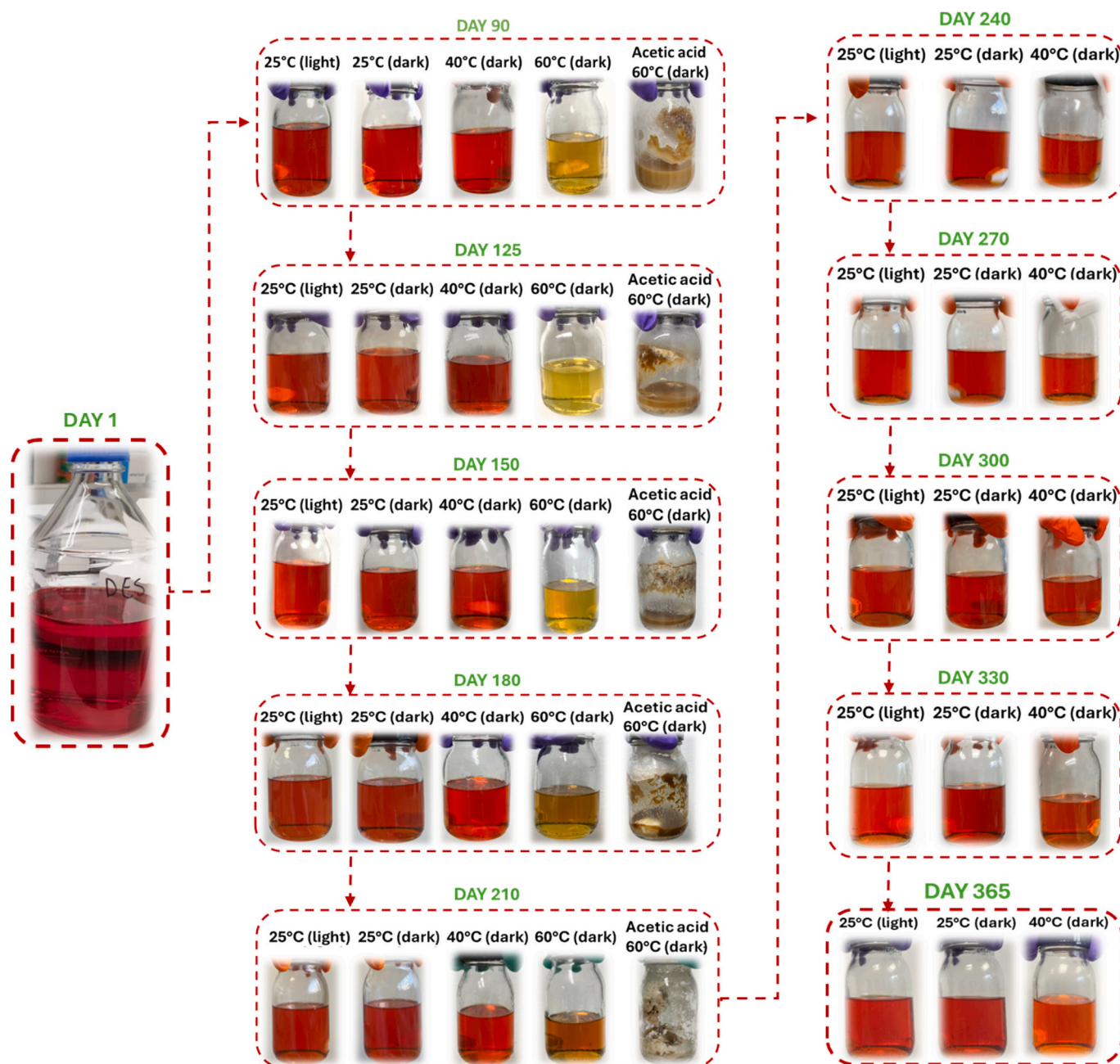


Fig. 7. Visual appearance of astaxanthin in DES and control formulations during storage under different conditions: 25 °C dark, 25 °C light, 40 °C dark, and 60 °C dark, illustrating colour retention and visual degradation over time. In the acetic acid extract (control) stability analysis was performed only at 60 °C.

3.4.1. Kinetic modelling of astaxanthin degradation

The kinetics of astaxanthin degradation during storage in the dark at 25, 40 and 60 °C were evaluated using first- and second-order kinetic models. Although astaxanthin degradation has often been described by first-order kinetics [29,35], a second-order kinetic model was also evaluated and compared. Fig. 8 shows the experimentally determined concentration profiles (3 replicates for each temperature) with those predicted by the first and second-order kinetic models. Table 4 presents the kinetic parameters and their corresponding confidence intervals, obtained through the regression analysis, and Table 5 reports the R^2 values calculated separately for each experimental dataset for both kinetic models.

Both the first- and second-order kinetic models provided a reasonable description of astaxanthin degradation, under the investigated storage conditions. However, the relative performance of the two

models depended on the storage temperature. At 25 and 40 °C, the second-order kinetic model provided a better fit to the experimental data than the first-order model, with R^2 values of 0.78 and 0.98, respectively, compared with 0.63 and 0.94 for the first-order model. In contrast, at the highest investigated temperature (60 °C), the first-order kinetic model provided a better description of the experimental data, as indicated by the higher R^2 value of 0.98 compared with 0.90 for the second-order model.

One possible explanation for the better performance of the second-order model at the lower temperatures is the potential formation of astaxanthin aggregates. Dai et al. [37] reported astaxanthin can form H- and J- aggregates with absorption maxima at approximately 388 and 565 nm, respectively, whereas monomeric astaxanthin exhibits an absorption maximum near 480–490 nm. Since the analytical method used in the present study estimated astaxanthin concentration based on

Table 3

Comparative analysis of astaxanthin stability in DES systems in the current study and literature.

Astaxanthin source	DES (molar ratio)	Stability conditions	Best results	Ref.
Shrimp shell waste	[N _{4,4,4,4}]Br:hexanol (1:2)	25, 40 and 60 °C (dark); 25 °C (light)	Astaxanthin in DES remained at ~47% after 1 month at 60 °C (dark), with complete degradation after 5 months. At 40 °C (dark), ~51% remained after 3 months (~81% loss after 12 months). At 25 °C, losses were ~50% (3 months) and ~81% (12 months) under light, and ~50% (9 months) and ~56% (12 months) in the dark.	This study
<i>Haematococcus pluvialis</i> (microalga)	DL-menthol:oleic acid (2:1), thymol:oleic acid (3:1), geraniol:oleic acid (13:1)	Light exposure (irradiation) for 13.5 h	Thymol: oleic acid-based extract retained 40% astaxanthin after 13.5 h while it was completely degraded in the remaining systems.	[33]
<i>Xanthophyllomyces dendrorhous</i> ATCC® 74219™ (yeast)	Thymol:octanoic acid, menthol, salol or camphor (1:1), menthol:octanoic acid (1:1), menthol: camphor (2:1)	25 °C for 42 h	Astaxanthin concentration decreased less than 5% after 42 h at 25 °C in thymol: salol.	[34]
Astaxanthin and astaxanthin ester standards	DES-based microemulsions (Tween and water) using DL-menthol:acetic acid, propionic acid, valeric acid or octanoic acid (1:2)	4 and 25 °C (dark) for 10 days	Astaxanthin and astaxanthin ester preserved with half-life (t _{1/2}) of 13 and 69 days, respectively, at 4 °C, in microemulsions containing menthol: octanoic acid.	[29]
<i>Haematococcus pluvialis</i>	DL-menthol:acetic acid or octanoic acid (1:2)	4, 25 and 40 °C (dark) for 15–30 days (temperature-dependent duration); 25 °C (light) for 30 days	Astaxanthin ester content in menthol: thymol remained at ~88% after 15 days at 40 °C in dark. Under light, degradation was ~11% over the first 8 days, with ~80% remaining after 30 days; under dark conditions, ~90% remained at day 30. In menthol: acetic acid, astaxanthin showed t _{1/2} of 7.04 days at 25 °C in the dark, decreasing to 1.75 and 1.52 days at 45 and 65 °C, respectively.	[30]
<i>Paracoccus carotinifaciens</i> (bacterium)	DL-menthol:thymol, camphor, α-terpineol, decanoic acid, oleic acid or linoleic acid; thymol: camphor or α-terpineol; camphor:α-terpineol, oleic acid or linoleic acid; α-terpineol:decanoic acid, oleic acid or linoleic acid (1:1)	25, 40 and 65 °C (dark and light) for 15 days	Under light, t _{1/2} was 5.55 days at 25 °C and 1.81–1.63 days at 45–65 °C.	[35]
Genetically modified <i>Picosynechococcus</i> sp. PCC 11901 (cyanobacterium)	Thymol:menthol (1:1); thymol:urea (5:1); thymol:phenylacetic acid (3:1); thymol:water (4.8:1); thymol:trimethylglycine (3:1); thymol: oleic acid (3:1); thymol:lauric acid (1:1)	25 °C (light) and 80 °C (dark) for 72 h	Astaxanthin content in thymol: water remained at ~60% after 72 h at 80 °C (dark); thymol: trimethylglycine was the only other system preventing complete degradation. Under light (25 °C), thymol: urea and thymol: Oleic acid retained >60% astaxanthin after 72 h, followed by thymol: lauric acid, thymol: water and thymol: trimethylglycine (>40%).	[36]

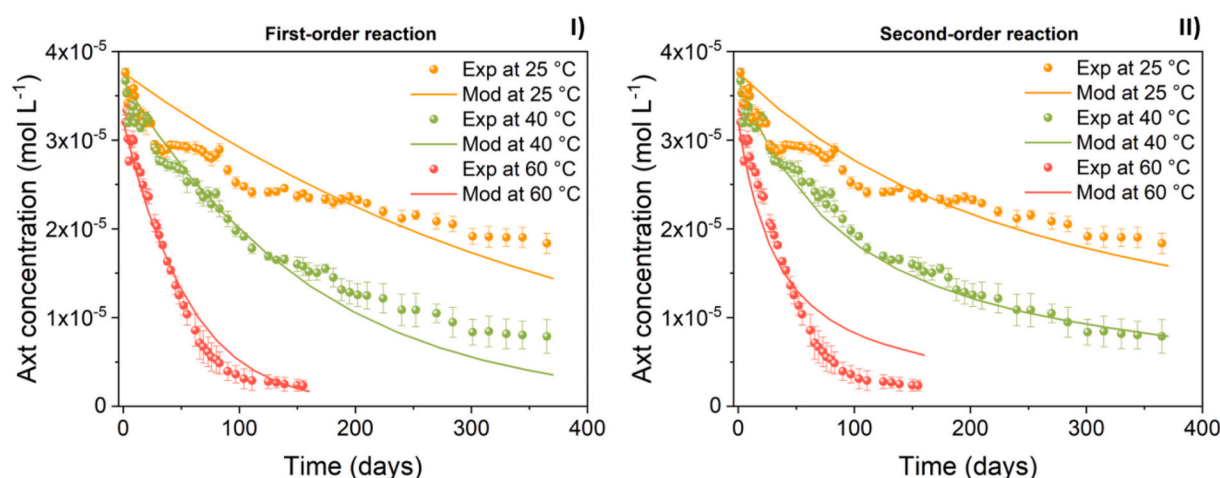


Fig. 8. Regression models of concentration profiles for astaxanthin during storage in the dark at 25, 40 and 60 °C. The numerical regression was performed for: I) first-order and II) second-order kinetic model. The dots with corresponding error bars indicate the experimental results determined during the stability test, while the lines show the concentrations predicted by each kinetic model.

absorbance at 482 nm, aggregation could potentially decrease the measured absorbance at this wavelength without necessarily corresponding to actual chemical degradation. Consequently, a reduction in the apparent monomeric astaxanthin signal could contribute to a concentration profile that is better described by second-order kinetics. However, aggregation was not directly investigated in the present study,

and this explanation therefore remains speculative and should be addressed in future studies.

The observed temperature dependent change in the kinetic model providing the best fit may indicate a change in the predominant degradation behaviour at elevated temperature. At 60 °C, the first order model provided a better fit than the second-order model, suggesting that

Table 4

Kinetic parameters determined by the regression models.

Reaction order	k at $T_{\text{ref}} = 25\text{ }^{\circ}\text{C}$	Ea [kJ Mol ⁻¹]	$t_{1/2}$ [days] at $T_{\text{ref}} = 25\text{ }^{\circ}\text{C}$
1	$(2.4 \pm 0.2) \times 10^{-3}$ [day ⁻¹]	46 ± 3	265 ± 19
2	100 ± 8 [L Mol ⁻¹ day ⁻¹]	52 ± 4	282 ± 20

Table 5Comparison of R² values for individual experiments based on regression of first- and second-order kinetic models.

Process parameter	First-order	Second-order
T [°C]	R ²	R ²
25	0.63	0.78
40	0.94	0.98
60	0.98	0.90

the process contributing to the observed astaxanthin loss may differ from those dominating at lower temperatures. However, the kinetic data alone do not provide sufficient evidence to confirm a change in the underlying degradation mechanism. A first-order model has also been reported to better describe astaxanthin degradation at elevated temperatures in flaxseed oil [38] and plant oil [39], which is consistent with the behaviour observed in the present study.

Although the apparent degradation behaviour varied with temperature, the calculated $t_{1/2}$ values provide a useful parameter for comparing the stability of astaxanthin under different storage conditions and with previously reported system. Therefore, the $t_{1/2}$ values obtained in the present study were compared with those reported previously in the literature. Li et al. [29] investigated the stability of astaxanthin and its corresponding ester in menthol-based DES. The first-order reaction rate constants determined at 25 °C for four tested DES ranged from 0.21 to 0.27 day⁻¹ for astaxanthin. The latter rate constants were approximately two orders of magnitude higher than the first-order rate constant obtained in the present study at the same storage temperature. Accordingly, the reported $t_{1/2}$ value was approximately 3 days at 25 °C, whereas the $t_{1/2}$ obtained in the present study was 265 days for the first-order kinetic model. Similarly, in a study employing a DL-menthol acid DES, $t_{1/2}$ values of 7.04 days were reported for dark storage at 25 °C [35], which suggests a 37-fold faster degradation than in the present study under similar storage conditions.

Overall, the substantially longer $t_{1/2}$ values observed in the present study indicate a considerably greater apparent stability of astaxanthin compared with the previously reported DES-based systems, particularly under elevated-temperature storage conditions.

4. Conclusions

This work demonstrates the potential of DES as selective and multifunctional media for the direct extraction and stabilisation of astaxanthin from crustacean shell waste. Among the DES evaluated, tetrabutylammonium bromide-based systems clearly outperformed choline chloride-based solvents, with [N_{4,4,4,4}]Br:hexanol emerging as the most effective solvent for both extraction efficiency and selectivity. Optimisation of the extraction conditions showed that the SLR was the most significant parameter affecting astaxanthin recovery, whereas extraction time had a comparatively lower impact. Under the selected conditions, namely a SLR of 0.4 g mL⁻¹ and an extraction time of 10 min, [N_{4,4,4,4}]Br:hexanol enabled the recovery of $80 \pm 3\text{ }\mu\text{g g}_{\text{shells}}^{-1}$ of astaxanthin, corresponding to $82 \pm 4\%$ of the pigment initially present in the biomass. At the same time, protein co-extraction was limited to approximately $23 \pm 1\%$, while minerals and chitin were not detectably extracted. This confirms the ability of the DES to selectively target

astaxanthin within the complex crustacean shell matrix. Beyond extraction, [N_{4,4,4,4}]Br:hexanol also provided substantial long-term stabilisation of astaxanthin. The pigment retained its characteristic orange colour after one year of storage, with the best stability observed at 25 °C in the dark, where approximately 44% of the initial astaxanthin remained after 365 days. Although higher temperatures and light exposure accelerated degradation, the DES system still performed markedly better than the acetic acid control, in which astaxanthin rapidly degraded. The improved stability is likely associated with the structured hydrogen-bond network and hydrophobic microenvironment of the DES, which may reduce pigment mobility, limit oxidative degradation, and provide a co-pigmentation-like stabilisation effect. The kinetic study revealed that at lower temperatures, the dominant mechanism for astaxanthin degradation is better described by a second-order model, while at higher temperatures (60 °C), first-order degradation is more pronounced. Overall, this study establishes [N_{4,4,4,4}]Br:hexanol as a multifunctional DES capable of selectively extracting and stabilising astaxanthin from shrimp shell waste. By integrating extraction and long-term stabilisation within a single solvent system under mild conditions, the proposed approach reduces downstream processing requirements, contributes to crustacean waste valorisation, and offers a promising route for the production of stable natural colourants from renewable marine residues.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seppur.2026.140547>.

Data availability

Data will be made available on request.

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