Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (*Carthamus tinctorius*)

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**A B S T R A C T**

A certain combination of natural products in the solid state becomes liquid, so called natural deep eutectic solvents (NADES). Recently, they have been considered promising new green solvents for foods, cosmetics and pharmaceuticals due to their unique solvent power which can dissolve many non-water-soluble compounds and their low toxicity. However, in addition to the features as solvents, the stabilisation ability of NADES for compounds is important for their further applications. In the study, the stability analysis demonstrates that natural pigments from safflower are more stable in sugar-based NADES than in water or 40% ethanol solution. Notably, the stabilisation capacity of NADES can be adjusted by reducing water content with increasing viscosity. The strong stabilisation ability is due to the formation of strong hydrogen bonding interactions between solutes and NADES molecules. The stabilisation ability of NADES for phenolic compounds shows great promise for their applications in food, cosmetic and pharmaceutical industries.

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1. Introduction

To extend the range of green solvents, we proposed natural ionic liquids and deep eutectic solvents (NADES) for applications in health-related areas such as food, pharmaceuticals and cosmetics (Choi et al., 2011; Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013a). NADES are liquid supermolecules made of natural primary metabolites bound together by inter-molecular interactions, particularly hydrogen bonding. They have several advantages over synthetic ionic liquids, e.g. their low costs, biodegradability, non-toxicity, sustainability, and simple preparation methods. Moreover, they show very good physicochemical properties as solvents: negligible volatility, liquid state even far below 0 °C, adjustable viscosity, wide polarity range, and high solubilisation strength for a wide variety of compounds (Dai et al., 2013a; Francisco, van den Bruinhorst, & Kroon, 2013). All those properties imply their potential for various types of applications in health-related areas. Up to now, they have been used in metabolite extraction (Usuki, Yasuda, Yoshizawa-Fujita, & Rikukawa, 2011), enzyme stability and enzymatic reactions (Corke, Srienc, & Kazlauskas, 2008; Kaar, Jesionowski, Berberich, Moulton, & Russell, 2003; Kragl, Eckstein, & Kafitzik, 2002). Few studies have been done to evaluate the stabilisation ability of NADES for natural products. Undoubtedly, it is essential to determine the stability of compounds in NADES in order to evaluate their applicability in all kinds of natural products processing.

Natural deep eutectic solvents (NADES) have a great potential as stabilising media for solutes, due to their unique physicochemical properties. NADES may occur in all organisms. NADES exist around the membranes of cells and are involved in the biosynthesis, solubilisation and storage of various poorly water-soluble metabolites and unstable compounds in cells (Choi et al., 2011). This provokes a number of ideas for applications of NADES. For example, carthamin is stable in the plant, which implies that, in safflower, NADES may stabilise carthamin as well as other pigments.

Safflower, the corolla from *Carthamus tinctorius* L. (Asteraceae) is used as a natural pigment, food additive, and cosmetic. Also, it is widely used as traditional medicine for cardiovascular diseases (Huang, Cui, & Ren, 1987; Shi & Liu, 2006). Antioxidant and neuroprotective properties have also been reported for safflower (Hirama, Takahashi, Komatsu, Kido, & Kasahara, 2009; Lee, Jeong, & Kim, 2009). The dried petals of safflower contain yellow and red pigments. Safflower yellow pigment is one of the few water-soluble yellow pigments found in nature. The major components of safflower yellow pigments are hydroxysafflower yellow A (HSYA), safflower yellow B and some other minor components, such as cartomin (Kazuma et al., 2000; Yin & He, 2000). HSYA has been reported to have an antithrombotic and neuroprotective effect (Wei et al., 2002).
Carthamin is the major red pigment in safflower and has antioxidant activity (Takahashi et al., 1982; Wang & Zheng, 2006). The basic structure of this pigment is a C-glucosyl quinochalcone (Jin et al., 2008; Kazuma et al., 2000).

However, carthamin is very unstable in an aqueous solution. It is usually extracted with an alkaline solution, but the red colour fades progressively to reddish orange, orange-yellow, yellow and light yellow (Saito, Yamamoto, & Miyamoto, 1992; Wang & Zheng, 2006). Carthamin has been reported to be more stable under alkaline than under acid and neutral conditions (Fatáhi, Carapetian, & Heidari, 2009). But it degrades rapidly when heated, having a half-life, under alkaline conditions, of 12.5 h at 25 °C and 0.75 h at 60 °C (Kim & Paik, 1997). Our former studies have proved that NADES have a high capacity for solubilising and extracting carthamin (Dai, Witkamp, Verpoorte, & Choi, 2013b; Dai et al., 2013a).

Natural deep eutectic solvents have totally different characteristics when compared with conventional solvents. The major components of NADES are natural primary metabolites, e.g. sugars, sugar alcohols, organic acids, amino acids and amines, which have several hydroxyl groups, carboxyl groups, or amino groups (Choi et al., 2011) Those groups give rise to hydrogen bonding interactions between two molecules, leading to highly structured viscous liquids (Zhang, Vigier, Royer, & Francois, 2012). Those liquids can, in turn, form additional hydrogen bonds with solutes, increasing their solubilisation ability, e.g. of phenolic compounds (Dai et al., 2013a,b). Natural deep eutectic solvents are like liquid crystals in which all molecules are arranged in a matrix with optimum inter-actions via inter- and intra- molecular H-bonding of the constituents. Studies are required to explore the full potential of NADES as solvents for food, medicines and cosmetics.

In this study, continuing our previous works to use NADES as a new extraction solvent, the stabilisation ability of typical NADES for carthamin was explored; the stabilisation mechanism was investigated, and NADES with high stabilisation ability for unstable phenolic compounds, such as carthamin, were developed. As an important factor, the effect of the water content in NADES on the stability of carthamin was investigated. This work will generate crucial knowledge for developing NADES with natural products to stabilise compounds especially unstable compounds, such as natural pigments and bioactive compounds.

2. Materials and methods

2.1. Chemicals, material and reagents

Carthamin was isolated in lab. Safflower was bought from Xinjiang province in China. The plant material was identified by one of the authors, Dr. Young Hae Choi, and a voucher specimen (NPL-carthamus-0913) was deposited in the Natural Products Laboratory, Institute of Biology, Leiden University. The dry plant material was ground into a powder in a blender with liquid nitrogen. Ethanol (EtOH), 40% EtOH, 90% GCH, 75%, 50% and 25% PMH with deionised water.

Carthamin solutions were prepared by dissolving carthamin in each solvent (water, 40% EtOH, NADES) with agitation for 30 min at room temperature. The samples were transferred into a 1.5 ml microtube, centrifuged at 1300 rpm for 20 min and then the supernatant was used for the test. Extraction was performed in sealed bottles with 50 mg of plant material and 3 ml of NADES or 40% ethanol, heating and stirring at 40 °C for 30 min. The sample was transferred into a 1.5 ml microtube, centrifuged at 1300 rpm for 20 min and then the supernatant was filtered through a 0.45 μm cellulose membrane. The resulting solutions were used to test the effect of storage, under ambient room conditions with sunlight, on the stability of the dissolved phenolic compounds.

2.3. Stability tests

The effects of heating, light, storage time, ambient conditions in sunlight, and water content in NADES on the stability of carthamin or safflower extracts were investigated with the methods described below. For thermal stability, carthamin solutions were put in glass vials with screw caps and placed in a preheated water bath at 80, 60 and 40 °C. Three tubes of each group were removed from the water bath after 10, 20, 40, 60, 80, 100 and 120 min and rapidly cooled to room temperature.

The effect of illumination from artificial light was determined with carthamin solutions at room temperature. Tubes with the test solutions were placed one metre below a lamp (TL 40 W, Philips) or covered with aluminium foil, and three tubes of each group were taken at days 0, 3, 7, and 15 for UV spectroscopy. The effect of storage time was investigated at –20 and 4 °C in the dark with carthamin solution and three tubes of each group were tested at days 0, 3, 7, 15, 30, and 60.

The effect of ambient conditions in sunlight was studied with carthamin solutions and safflower extract solutions. Each solution was exposed to room conditions in the daylight and three samples of each group were removed at days 0, 3, 7, 15 for HPLC analysis.

The effect of water content in NADES on the stability of carthamin was investigated with two NADES (PMH and SuCH) at 4 °C and –20 °C in the dark. PMH and SuCH were used with 0%, 25%, 50%, and 75% (v/v) water. All stability tests were done in triplicate.

2.4. Apparatus and analysis

A UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan) was used for the stability test of carthamin, at a wavelength of 520 nm. Extracts were analysed with an Agilent 1200 HPLC-DAD on a Phenomenex Luna C18 (2) (4.6 μm × 250 mm, particle size 5 μm) column. The mobile phase consisted of 0.5% H3PO4 (A) and acetonitrile (B) in a linear gradient programme as follows: 5–11% B (0–10 min), 11–14% B (10–16 min), 14% B (16–23 min), 14–20% B (23–30 min), 20–35% B (30–70 min), 35–60% B (70–80 min) at a flow rate of 1.0 ml/min (Wang, Yang, & Fu, 2008). The injection volume was 10 μl. Chromatograms were recorded at 520, 403, and 280 nm. FT-IR spectra, over the range 4000 to 300 cm−1, were registered at room temperature (25 °C), using a Bruker FT-IR spectrometer. The pH of the diluted NADES with 90% (v/v) deionised water was tested with pH indicator paper (Merck, Darmstadt, Germany).

2.5. Data analysis

For calculation of kinetic parameters of carthamin degradation at high temperature, the first-order reaction rate constants (k) and half-lives (t1/2), for degradation of 50% of carthamin, were calculated by the following equations (Kircı, Özkam, & Cemeroğlu, 2007):
In(c/c_0) = −kt

\[ t_{1/2} = -\ln(0.5)/k \]

where \( c/c_0 = A/A_0 \), \( A_0 \) is the initial absorption of diluted carthamin and \( A \) is the absorption value of diluted carthamin after heating time \( t \) at a given temperature.

3. Results and discussion

3.1. General

To test the effects of solvent features, such as viscosity, polarity and composition of NADES (acidic or basic), on their stabilisation ability, the following five typical NADES were selected: proline-malic acid (PMH), lactic acid–glucose (LGH), glucose-choline chloride (GCH), sucrose-choline chloride (SuCH), and xylitol-choline chloride (XoCH) (Table S1). Physical properties of each NADES with diverse compositions show unique pH, viscosity and polarity (Dai et al., 2013a). LGH and PMH are characterised as acidic, with a pH 3 when diluted with 90% (v/v) of water, and the other NADES after dilution have pH values from 6 to 7 (Table S1). Regarding their viscosity, PMH, SuCH and GCH are the most viscous, followed by XoCH, while LGH has the lowest viscosity. LGH is the most polar of the studied NADES, whereas PMH is similar to water, and sugar/sugar alcohol-choline is slightly less polar than water. It was reported that 40% ethanol has the highest extraction ability for the pigments from safflower (Zhang, Wang, & Liu, 2009), while water is a common general extraction solvent for the safflower yellow pigment (Wang, Zhang, Jin, & Su, 2010; Zhu, Pan, Jin, & Yang, 2008). Thus, the stabilisation ability of NADES was investigated using both water and 40% ethanol as references.

3.2. Stability of carthamin during heating

As an example, XoCH was selected in this experiment because carthamin is most soluble in this NADES (Dai et al., 2013a). Temperature is an important factor in the stability of carthamin. The degradation rate of carthamin in aqueous solution was reported to increase at high temperatures by Fatahi et al. (2009). This was also observed in our experiments, in which the degradation rate of carthamin increased with increasing temperature (Fig. 1). However, carthamin was much more stable in XoCH than in water. At all tested temperatures, the degradation rate of carthamin in XoCH was much slower than in water. At 60 and 40 °C, first-order kinetics were observed for the degradation of carthamin in both XoCH and water, which is in agreement with a previous report on the degradation of carthamin in aqueous solution (Kim & Paik, 1997). At 60 °C, the half-life \( (t_{1/2}) \) of carthamin in XoCH was more than twice than in water (Table S2). Furthermore, the \( t_{1/2} \) of carthamin is 5 times longer in XoCH and 8 times longer in water at 40 °C if compared to 60 °C. Therefore, compared with water, XoCH shows a clear protective effect against thermal degradation.

3.3. Stability of carthamin in light

The stability of carthamin in light was investigated at room temperature, exposing a solution to 24 h of light under a daylight lamp over a 15-day period, compared with in the dark. The effect of light on the stability of carthamin differed according to the solvents (Fig. S1). The degradation curves of carthamin in light and in the dark overlapped in three of the solvents, GCH, SuCH and water, implying that light has no obvious effect on the stability of carthamin in those three solvents at room temperature for 15 days (Fig. 2a). With light, carthamin degraded faster than in the dark when dissolved in LGH and 40% ethanol, and especially more in PMH, suggesting that light accelerates the degradation of carthamin in these solvents at room temperature (Fig. 2b).

As regards different solvents, comparing the degradation curve of light-exposed solutions of carthamin in 90% GCH and 75% SuCH, the stability was very much higher than that in 40% ethanol and water solution. Thus, SuCH and GCH are good solvents for carthamin in that exert a higher stabilisation effect than do conventional solvents and show no obvious effect from light.

3.4. Stability of carthamin according to storage time

At −20 °C, carthamin was stable in all tested solvents over a 7-day period. After 15 days, it was still stable in SuCH, and less than 10% degradation occurred in other NADES (PMH, 90% GCH, LGH), while substantial degradation was observed in water and 40% ethanol (Fig. 3a). At 4 °C, carthamin remained stable in SuCH over 1 month, while it showed degradation in all other solvents, including NADES. The stability of carthamin solution decreases in the following sequence: SuCH > GCH > LGH > PMH = 40% EtOH > water (Fig. 3b). In particular, at 4 °C the degradation of carthamin was below 5% in the NADES in the first 3 days, while it was near 15% in

Fig. 1. Stability of carthamin in xylitol-choline chloride (a) and water (c) at high temperature (a) 80 °C, (b) 60 °C and (c) 40 °C. Results are based on triplicates.

Fig. 2. Degradation curve of carthamin standard in light (L) from lamp and in the dark (D) at room temperature over a 15-day period in two groups of solvents (group a: 90% glucose-choline chloride (GCH), 75% sucrose-choline chloride (SuCH), and water; group b: proline-malic acid (PMH), lactic acid–glucose (LGH) and 40% (v/v) ethanol). Results are based on triplicates.
ethanol and 38% in water. Comparing −20 and 4 °C, the degradation of carthamin was increased from 35% to 61% in ethanol and from 16% to 26% in GCH after 30 days. Thus, NADES also exert a stabilising effect on carthamin during storage. Therefore, carthamin can be preserved in SuCH at 4 °C for at least 1 month and at −20 for at least 3 months.

Carthamin is more stable in SuCH and GCH than in PMH and LGH under all aforementioned conditions (Table S1). The big difference among those NADES is among their viscosities: SuCH and GCH have a much higher viscosity than have PMH and LGH. Thus, the stabilisation ability of NADES may have a direct relationship to their viscosity. Moreover, the viscosity of NADES is affected by temperature inversely, so that the viscosity of NADES decreases with increased temperature, which may partly explain why the stability of carthamin decreases with increased temperatures. For instance, in the case of PMH and LGH, the stability of carthamin decreased significantly when the temperature increased from −20 to 4 °C. The high viscosity at low temperature decreases the movement of molecules, allows stable molecular interactions between solvents and carthamin (see Section 3.6), and therefore possibly reduces the degradation of carthamin.

3.5. Stability of carthamin and safflower extract under ambient conditions in sunlight

The red coloured safflower was selected to explore the stabilising ability of NADES for aromatic pigments with different polarities under ambient conditions in sunlight. The retention times, in HPLC profile, corresponded well with their polarity. The HPLC profile at 280 nm of the safflower extract shows the presence of many phenolic compounds (Dai et al., 2013b). Because of the complexity derived from overlapping and minor compounds, three typical peaks with different retention times, (Tr) were selected to evaluate the stabilising ability of NADES for phenolic compounds in safflower. Three representative peaks were selected: hydroxysafflor yellow A (HSYA) (Tr = 21.9 min), cartormin (Tr = 40.0 min) and carthamin (Tr = 72.9 min). The chosen compounds cover the compounds from polar to less polar and are major active metabolites or pigments in the safflower extract, which should reflect the stabilisation ability of NADES for phenolic compounds in terms of polarity. The stabilities of the safflower extract and carthamin standard in NADES were investigated under ambient conditions in sunlight over a 15-day period.

The results show that the degradation of carthamin standard was very different in each solvent (Fig. 4). The stability of carthamin decreased in the following sequence: 90% GCH > 75% SuCH > LGH > 40% EtOH > PMH = water (Fig. 4a). The carthamin in the safflower extract solution showed a similar stability profile to the carthamin standard after 15 days (Fig. 4b). The degradation of carthamin was found to be 25% in 90% GCH 3 days after treatment, and 60% in water and 40% ethanol. Thus, the stability of carthamin is markedly improved in 90% GCH as compared with that in water and EtOH (40%).

The behaviours of HSYA and cartormin are very different from that of carthamin. HSYA was stable in 90% GCH and 75% SuCH, and showed a 5% degradation in water and 40% ethanol in 15 days. However, it degraded rapidly in LGH and 75% PMH (Fig. 4c). Cartormin was also stable in 90% GCH and 75% SuCH, exhibited around 10% in water and 40% ethanol in 15 days, and also degraded dramatically in LGH and 75% PMH (Fig. 4d). Light has been reported to affect the stability of safflor yellow extract (main components, including HSYA and cartormin), in buffer solution (Fatahi et al., 2009); the main components of safflor yellow, HSYA and cartormin, however, are more stable in 90% GCH and 75% SuCH than in water and 40% ethanol in our studies. This indicates that 90% GCH and 75% SuCH are far better solvents for storage of phenolic compounds in safflor yellow under ambient conditions and better than water and 40% ethanol. Ultimately, SuCH and GCH are promising as solvents and colour protectors for safflower yellow extract when applied in the food or pharmaceutical industry.

Our results reveal that carthamin (red pigment), HSYA and cartormin (major components of safflor yellow) are more stable in GCH and SuCH than in PMH, LGH, water or 40% ethanol. Sugars (xylose, glucose, sucrose, fructose, lactose) were reported to protect the colour of safflor yellow B (a major component of safflor yellow) at high temperature (Saito & Murata, 1994). Therefore, in the first place, it could be possible that the sugar component in NADES may play an important role in stabilising the safflower extract.
tract under ambient conditions, probably due to hydrogen bonding with solutes. Secondly, pH is reportedly an important factor for the degradation of safflower extracts (Saito & Mori, 1994). Safflower yellow is more stable in acidic (pH 2–6) than basic solutions (Fatahi et al., 2009; Yoon et al., 2003; Zhu et al., 2008). LGH and PMH contain acidic ingredients and they are more acidic than SuCH and GCH when diluted with 90% (v/v) water. However, HSYA and cartormin are much more stable in GCH and SuCH than in LGH and PMH. Thus, there is no relationship between the stability of HSYA and cartormin and the presence of acids in NADES. Carthamin, on the other hand, is more stable in GCH and SuCH than in PMH and LGH, which is in agreement with the report that carthamin is more stable in basic than acidic aqueous solution (Fatahi et al., 2009; Kim & Paik, 1997). Lastly, the direct relationship between the stabilising ability of NADES and their high viscosity is confirmed. The viscosity of NADES is greatly affected by their water content (Dai et al., 2013a) so that the water content may affect the stabilising ability of NADES, as demonstrated below (Section 3.6). All things considered, safflower extracts are more stable in high viscous non-acid-containing NADES.

3.6. Stability of carthamin in NADES with different water contents

The effect of the water content (in the form of water percentage) on the stability of carthamin was investigated in two NADES (SuCH and PMH) at 4 °C and −20 °C. Both are highly viscous (Dai et al., 2013a), but have different compositions and acidic properties after dilution. Their viscosity is affected by the water percentage and temperature. As discussed previously, the viscosity of NADES affects their solubilisation ability according to their dilutions with water and may also affect their stabilisation capacity. Therefore, it is necessary to evaluate the effect of the water content on the stability of compounds in NADES for their application in dissolving and storage of compounds.

The results showed that the water content of NADES plays an important role in the stability of carthamin. At 4 °C, carthamin was stable in SuCH, but less stable in water-diluted SuCH, with a decreasing stability from SuCH: H₂O (3:1), to SuCH: H₂O (1:1), and SuCH: H₂O (1:3) (Fig. 5a). In PMH, the stability of carthamin decreased in the following sequence: PMH > PMH: H₂O (3:1) = PMH: H₂O (1:1) > PMH: H₂O (1:3) (Fig. 5b). After 15 days, the level of carthamin in pure SuCH was still the same, but decreased by 25% in SuCH:H₂O (3:1), and by 75% in both SuCH:H₂O (1:1) and SuCH:H₂O (1:3). Therefore, the stabilisation ability of NADES increases with increasing viscosity (low water content). At −20 °C, the effect of the water content in NADES on the stability of carthamin was lower and became visible after 7 days of storage (Fig. 5c, d). Thus, lower water content in highly viscous NADES at low temperature is good for the stability of carthamin.

3.7. Mechanism of the stabilising ability of NADES for phenolic compounds

FT-IR was recorded for a typical phenolic compound, quercetin, dissolved in GCH (Fig. 6). The spectra show different absorption bands of the –C=O and aromatic ring, as well as the carboxyl group, from quercetin in the solid state and dissolved in GCH. The stretching vibration absorption band of the –C=O of quercetin shifted from 1168 to 1164 cm⁻¹, indicating that the hydroxyl groups in quercetin donate protons to form hydrogen bonds with solvent molecules. The deformation vibration absorption bands of the –C=O in quercetin shifted from 1355 to 1369 cm⁻¹ and from 1210 to 1200 cm⁻¹, which confirms new hydrogen bonds formation between quercetin and GCH and also implies that quercetin has a different conformation in GCH from that in the solid state. The shift of the characteristic band of the aromatic ring of quercetin from 1615 to 1600 confirmed the structure deformation of quercetin in GCH (Heneczowski, Kopacz, Nowak, & Kuźniar, 2001). In addition, the downward shift of C=O from 1669 cm⁻¹ to 1654 cm⁻¹ indicates H-bonding (C=O–HO) between the carbonyl group of quercetin and solvent hydroxyl groups (glucose and choline). All the above spectral characters of quercetin reveal the existence of multi H-bond interactions between quercetin and NADES, which is in agreement with the interaction signals in the NOESY spectrum of quercetin in XoCH (Dai et al., 2013a).

The H-bond interactions between solute and molecules of NADES provide an explanation for the high stabilising ability of

Fig. 5. Stability of carthamin standard in sucrose-choline chloride (SuCH) and proline-malic acid (PMH) with different percentages of water in the dark over a 15-day period at (a) and (b) 4 °C and (c) and (d) at −20 °C: 1. 100% SuCH; 2. 75% SuCH; 3. 50% SuCH; 4. 25% SuCH; 5. 100% PMH; 6. 75% PMH; 7. 50% PMH; 8. 25% PMH. Results are based on triplicates.

Fig. 6. FT-IR spectra of (1) glucose-choline chloride (2) quercetin in glucose-choline chloride and (3) quercetin.
the sugar-based NADES such as SuCH. It was reported that the water extract of safflower is stable under acid conditions (pH 2–6), in which most phenolic compounds are in the neutral form. Further studies showed that sucrose, glucose, starch and the common ions Ca$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$, can improve the stability of the extract (Zhu et al., 2008). All these examples confirm the conclusion that the formation of hydrogen bonds or chelation can stabilise the structures of phenolic compounds.

All things considered, we hypothesise that the NADES solutions of safflower extract are, in fact, liquid crystals in which the pigment molecules are fixed in the crystals. Degradation by oxidation thus only occurs on the surface of the liquid crystals. Melting the crystals, by increasing the temperature or adding water, allows the phenolics to diffuse freely through the system and thus increased oxidation will occur.

4. Conclusions

In this study, some typical NADES were demonstrated to be better solvents for the stabilisation of phenolic compounds than are general solvents. The NADES improve the stability of carthamin under various conditions, such as high temperature, light and storage time, if compared to water and 40% ethanol. Furthermore, the higher colour stability of carthamin, as well as of the other two phenolic compounds in the safflower extract, was also observed in NADES when exposed to sunlight under ambient conditions.

The high stabilising ability of NADES seems to be correlated with the strong hydrogen bonding interactions between solutes and solvent molecules. High viscosity of sugar-based NADES with low water content allows stable molecular interactions and plays an important role in the stabilising effect of NADES for phenolic compounds. NADES are like liquid crystals in which the pigment molecules are fixed in the crystals.

The high stability of the three typical phenolic compounds from safflower in sugar-based NADES throws new light on the stabilising ability of NADES for phenolic compounds. Further studies on the stabilisation effect in other sugar-based NADES and NADES made of organic acids may not only provide further understanding of the principle of the stabilising ability of NADES, but also lead to novel applications of NADES in the food and pharmaceuticals industry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.02.155.

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