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Microwave-assisted extraction of soluble sugars from banana puree with natural deep eutectic solvents (NADES)

Analía Verónica Gómez[a,b], Carmen Cecília Tadini[a,b,*], Atanu Biswas[c], Megan Buttrum[c], Sanghoon Kim[c], Veera M. Boddu[c], H.N. Cheng[d,**]

[a] University of São Paulo, Escola Politécnica, Chemical Engineering Dept., Main Campus, São Paulo, 05508-010, SP, Brazil
[b] University of São Paulo, FoRC/NAPAN – Food Research Center, São Paulo, SP, Brazil
[c] USDA Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL, 61604, USA
[d] USDA Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA, 70124, USA

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A B S T R A C T

Over-ripe bananas serve as a good source for non-starch polysaccharides (NSP), which can be used as a functional food ingredient and represent an opportunity for waste utilization. However, ripe banana contains a large amount of sugars, which are undesirable and need to be removed. Traditional extraction methods using alcoholic solvents have many drawbacks. This study aims to use natural deep eutectic solvents (NADES) as new and eco-friendly solvents for the extraction of soluble sugars from ripe bananas. Thirty NADES were characterized and screened, and four of them were selected as most appropriate solvents to remove soluble sugars from banana puree with the help of microwave-assisted extraction. The effects of temperature, time, and quantity of water added to NADES were evaluated. In all cases, NADES were shown to be more effective than conventional solvents (water and ethanol). Among the four NADES, malic acid:beta-alanine:water (1:1:3, molar ratio) with 30 g/100 g of water (25 °C, 30 min) was found to be the most effective in the extraction of soluble sugars from banana puree. Thus, NADES can be considered a highly efficient extraction medium for fruits (such as bananas) and can replace conventional extractions using harsher organic solvents like ethanol.

1. Introduction

Brazil is the fourth largest banana producer in the world (6.8 million tons per year), with production concentrated in the Northeast region (FAO, 2016). Although the production level is high, a lot of bananas are lost at the post-harvest stage and rejected fruits are normally improperly disposed (Villaverde et al., 2013). Because of this, it would be desirable to use them as sources for innovative food ingredients that can rise their commercial value.

Non-starch polysaccharides (NSP) are among the main components of the cell walls of many edible plants and grains. They are not susceptible to α-amylase and are only used through the fermentation microbiota in the colon. NSP present in ripe bananas are classified as dietary fibers and can produce beneficial effects on human health (Cordenunsi, Shiga, & Lajolo, 2008). Isolation of NSP from different food matrices has been studied by several authors as functional ingredients in the human diet (Chen, Ma, Liu, Liao, & Zhao, 2012; Kumar, Sinha, Makkar, de Boeck, & Becker, 2012).

It is important to note that rejected bananas can be used as useful raw materials and their degree of maturation does not influence the quantity of the NSP, which can be obtained even from over-ripe fruits. Large amounts of sugars in ripe banana are not desirable in a NSP-isolated product which is targeted as a food ingredient. Therefore, a potential opportunity is to isolate the NSP with the least quantity of soluble sugars for use as a food ingredient. Traditional extraction methods use alcohols as solvent and have several drawbacks: they are time-consuming, laborious, employ large amounts of solvent, and have low selectivity and/or low extraction yields.

Green chemistry aims to preserve the environment by using the minimum quantity of non-hazardous materials (green solvents) and eco-friendly solubilization techniques. In this context, natural deep eutectic solvents (NADES) have been paid great attention to replace many of the current organic solvents. NADES can be obtained by mixing natural materials like simple sugars (glucose and fructose), low molecular weight acids (malic, citric, and lactic acid), choline chloride, and urea. These compounds are capable of self-association, often through

* Corresponding author. University of São Paulo, Escola Politécnica, Chemical Eng. Dept., São Paulo, SP, 05508-010, Brazil.
** Corresponding author. USDA Agricultural Research Service, Southern Regional Research Center, New Orleans, LA, 70124, USA.
E-mail addresses: catadini@usp.br (C.C. Tadini), hn.cheng@ars.usda.gov (H.N. Cheng).

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hydrogen bond interactions, and the resulting eutectic mixture has a melting point that is lower than those of the individual components (Del Monte, Carriazo, Serrano, Guitiérrez, & Ferrer, 2014). These new and environmentally friendly solvents have attractive physicochemical properties, such as chemical and thermal stability, no flammability, high electrical conductivity, and a good solubilizing capacity for a number of organic compounds (Zhang, De Oliveira Vigier, Royer, & Jérôme, 2012). Electrical conductivity is considered an attractive physicochemical property of NADES solvents because it is correlated with their viscosity. Most NADES with a low conductivity are highly viscous. The high viscosity of NADES hinders the efficiency of them as extraction solvents due to the low mass transport efficiency. In order to remove this hindrance, some dilutions with water can be done to improve the viscosity of NADES. Through dilution with water, the interactions between the components become weaker leading to a decrease of the viscosity of the NADES and as a result the conductivity increases by 10–100 times. These results have been demonstrated by Dai, van Spronsen, Witkamp, Verppoorte, and Choi (2013).

NADES have been employed in a wide range of applications, including bioenergy (Kleiner, Fleischer, & Schörken, 2016; Zhang et al., 2016), antimicrobial uses (García-Argüelles et al., 2013; Wikene, Rukke, Bruzell, & Tønnesen, 2017) and separation and extraction of target compounds from natural products (Bosiljkov et al., 2017; Huang et al., 2017; Li et al., 2016).

Because of the newness of NADES, not much work has been done to use them for the extraction of selected components from foods. The purpose of this work was the extraction of soluble sugars from ripe banana puree with NADES in combination with microwave-assisted extraction (MAE), in order to obtain a purified non-starch polysaccharide matrix, which could be used as a functional food ingredient.

2. Materials and methods

2.1. Characterization of raw material

Bananas were acquired from a local supplier and were kept at 20 °C in a conditioned room. Samples were selected, washed, peeled and cut according to Tribess et al. (2009). Immediately the slices were immersed for 5 min in a citric acid solution (1 g/1 L) to delay the enzymatic darkening of the fruit. Then, the slices were triturated with a blender to obtain banana puree.

Firmness analyses were conducted in the center of the fruit through a penetration test with a texture analyzer TA.XT plus (Stable Micro System, Surrey, UK) to assess the degree of ripeness (Tribess et al., 2009). A cylindrical steel probe (TA55, 5 mm diameter) was used at a rate of 1.0 mm s^{-1} and a penetration depth of 20 mm. For moisture content, banana puree (3–5 g) was dried at 70 °C in an Isotemp® vacuum oven, model 285A (Fisher Scientific, Hampton, NH, USA) until constant weight. Soluble solids were measured using an ABBE 3L refractometer (Bausch & Lomb, Rochester, NY, USA) at 20 °C. The measurement of titratable acidity was carried out according to the Zenebon and Pascuet (2005). Total sugars were determined by the phenol-sulfuric acid method according to Masuko et al. (2005).

2.2. Preparation and characterization of NADES

All chemicals were purchased from Sigma (Sigma-Aldrich Inc., St. Louis, MO, USA). Several NADES were prepared and tested by combining different compounds at different molar ratios, times and melting temperatures. Due to the high viscosity of NADES, it was necessary to add small amounts of water during preparation. NADES components were heated and mixed in a Reacti-Therm III heating/stirring module (Thermo Fisher Scientific, Rockford, IL, USA). Measurements of physicochemical properties were done on NADES diluted with different percentages of water (0–90 g/100 g). The electrical conductivity was measured at 25 °C using a S470 SevenExcellence™ conductivity meter equipped with an InLab731 ISM sensor (Mettler Toledo, Columbus, OH, USA). Soluble solids were measured at 20 °C with an ABBE 3L refractometer (Bausch & Lomb, Rochester, NY, USA). The dynamic viscosity was measured at 25 °C using an ARES LS-1 control strain rheometer (TA Instruments, New Castle, DE, USA). The density was measured at 40 °C using a vibrating tube density meter, model DMA™ 4500 M (Anton Paar, Graz, Austria). The water activity was measured at 25 °C with an AQUA-LAB Water activity meter, Series 3 TE (Decagon Devices Inc., Pullman, WA, USA).

Table 1
3^3 full factorial design layout. Factor A: Temperature [°C], Factor B: time [min], Factor C: quantity of water added to NADES [g/100 g].

Run	Design matrix					
	Coded Factors			Uncoded Factors		
	Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	**Factor A (x_1)**	**Factor B (x_2)**	**Factor C (x_3)**
1	−1	−1	0	25	5	40
2	−1	1	0	25	30	40
3	1	−1	0	70	5	40
4	1	1	0	70	30	40
5	0	−1	−1	50	5	30
6	0	−1	1	50	5	50
7	0	1	−1	50	30	30
8	0	1	1	50	30	50
9	−1	0	−1	25	15	30
10	1	0	−1	70	15	30
11	−1	0	1	25	15	50
12	1	0	1	70	15	50
13	0	0	0	50	15	40
14	−1	−1	−1	25	5	30
15	1	−1	−1	70	5	30
16	−1	1	−1	25	30	30
17	1	1	−1	70	30	30
18	−1	−1	1	25	5	50
19	1	−1	1	70	5	50
20	−1	1	1	25	30	50
21	1	1	1	70	30	50
22	−1	0	0	25	15	40
23	1	0	0	70	15	40
24	0	−1	0	50	5	40
25	0	1	0	50	30	40
26	0	0	−1	50	15	30
27	0	0	1	50	15	50

Table 2
Moisture, soluble solids, titratable acidity and firmness of the bananas after 14 days of storage.

Assay	Result	Literature data
Moisture [g/100 g]	78.58 ± 0.10	–
Soluble solids [°Brix]	19.23 ± 0.15	20.9[a]
Titratable acidity [g/100 g]	2.74 ± 0.08	2.66[a]
Firmness [N]	0.801 ± 0.017	0.804[a]

[a] Ditchfield and Tadini (2002)

2.3. Experimental design and statistical analysis

A 3^3 full experimental design was used to evaluate the effects of the variables temperature (X_1; °C), time (X_2; min) and quantity of water added to NADES (X_3; g/100 g), on the extraction of soluble sugars (SS) under MAE conditions. Three replicates at the central point were carried out to estimate the random error and to examine the presence of curvature in the response surfaces. Three factorial level coded for low, medium and high settings (−1, 0 and + 1, respectively) were considered for the three independent variables. On the basis of preliminary assays, 25, 50 and 70 °C were chosen as temperature levels and 5, 15 and 30 min were selected as extraction time levels. Regarding the

Table 3
Different combinations of natural deep eutectic solvents (NADES): Molar ratio, temperature (*T*), and time (*t*) to obtain NADES solvents.

Component 1	Component 2	Component 3	Molar ratio	*T* [°C]	*t* [min]	Obs.	Selected NADES	Abb.
Glucose	Choline chloride	Water	2:5:5	70	60	Colorless liquid		
Fructose	Choline chloride	Water	2:5:5	70	60	Colorless liquid		
Glucose	Choline chloride	Water	2:4:4	70	120	Colorless liquid		
Glucose	Choline chloride	Water	2:3:3	70	120	Colorless liquid	✓	Glu:CC
Fructose	Choline chloride	Water	2:3:3	70	60	Colorless liquid	✓	Fru:CC
Citric acid	Glucose	Water	1:1:2	80	120	Colorless liquid	✓	CA:Glu
Citric acid	Fructose	Water	1:1:2	75	120	Yellowish liquid	✓	CA:Fru
Citric acid	Glucose	Water	2:1:2	80	150	Solid		
Citric acid	Choline chloride	Water	1:1:2	60	60	Colorless liquid	✓	CA:CC
Citric acid	b-alanine	Water	1:1:3	60	60	Colorless liquid	✓	CA:BA
Urea	Glucose	Water	2:1:2	70	60	Colorless liquid		
Urea	Fructose	Water	2:1:2	70	60	Colorless liquid		
Urea	Glucose	Water	1:1:2	70	60	Colorless liquid	✓	U:Glu
Urea	Fructose	Water	1:1:2	70	60	Yellowish liquid	✓	U:Fru
Urea	Fructose	Water	1:1:2	60	90	Yellowish liquid		
Urea	Glucose	Water	1:2:2	80	120	Solid		
Malic acid	Glucose	Water	1:1:3	70	60	Colorless liquid	✓	MA:Glu
Malic acid	Glucose	Water	1:1:3	60	120	Solid		
Malic acid	Fructose	Water	1:1:3	60	60	Yellowish liquid	✓	MA:Fru
Malic acid	Glucose	Water	1:1:2	80	90	Colorless liquid		
Malic acid	Fructose	Water	1:1:2	60	90	Yellowish liquid		
Malic acid	Choline chloride	Water	1:1:2	60	60	Colorless liquid	✓	MA:CC
Malic acid	b-alanine	Water	1:1:3	60	60	Colorless liquid	✓	MA:BA
Tartaric acid	Glucose	Water	1:1:2	80	120	Solid		
Tartaric acid	Glucose	Water	1:1:3	80	120	Solid		
Tartaric acid	Glucose	Water	1:2:3	80	120	Solid		
Tartaric acid	Fructose	Water	1:2:3	80	120	Solid		
Oxalic acid	Glucose	Water	1:1:2	80	120	Solid		
Glutamic acid	Glucose	Water	1:1:3	80	120	Solid		
Lactic acid	Glucose	Water	5:1:3	80	60	Colorless liquid		

quantity of water added to NADES (QW), some authors (Gutierrez, Ferrer, Mateo, & del Monte, 2009; Dai, Witkamp, Verpoorte, & Choi, 2015) found that the supramolecular complex structures of NADES were preserved while the content of water was below 50 g/100 g, but further dilutions produced a solution of the individual components in water. On the basis of these results, quantities of 30, 40 and 50 g/100 g of water were added to NADES.

The quantity of SS extracted from banana puree (Y_i; g/100 g) was taken as the response of the experimental design. A total of 27 experimental runs were required for analyzing the interaction of each level on formulation characters (Table 1).

The data obtained were presented as means of at least two replicate determinations ± standard deviation. Results were submitted to analysis of variance to compare means with one-way ANOVA and Tukey post hoc test ($p < 0.05$), using software Statgraphics Centurion version XVI.I (StatPoint Technologies Inc., Warrenton, VA, USA). To evaluate the factorial design with center point the same software was used for statistical treatment of the results.

2.4. Extraction of soluble sugars with NADES by microwave-assisted extraction (MAE)

Soluble sugars (SS) from banana puree were extracted in an ETHOS 1600 Advance Microwave Labstation (Milestone, Sorisole, Italy) with a fixed frequency of 2.45 GHz and a maximum output power of 900 W. The real-time temperature inside the reactor was measured and controlled with a thermometer probe sensor. Banana puree (3 g) was weighed into a vessel, and SS were extracted with NADES (30 g) at different temperature (25, 50, 70 °C), time (5, 15, 30 min), and QW (30, 40, 50 g/100 g). Samples were heated from room temperature until the set temperature in 3 min and kept at the selected temperature during the established time. After extraction, samples were centrifuged at 4 °C for 20 min in a Heraeus Multifuge X1R centrifuge (Thermo Fisher Scientific, Langenselbold, Germany) at 10,000 × g to separate the supernatant from the residue. An aliquot of each supernatant was analyzed for total sugars by the phenol-sulfuric acid method (Masuko et al., 2005).

3. Results and discussion

This work was conducted in two main stages. The first stage was to identify the appropriate NADES to be used as solvents in removing soluble sugars from banana puree. The second stage was to use the selected NADES in combination with MAE to improve the extraction of the SS.

3.1. Characterization of raw material

The physicochemical parameters and the firmness of the raw material in relation to the optimum degree of maturation necessary to obtain a homogeneous banana puree were standardized. The evolution of banana ripening was monitored throughout 14 days. At day 0, the firmness presented a value of 2.630 N (green banana), this value decreased during the maturity process until it reached a value of 0.801 N at the end of the storage period (yellow banana with large dark brown spots). This phenomenon is related to the ripening of bananas during their storage, where the firmness of the fruit decreases due to the degradation of pectins and the hydrolysis of the starch into simple sugars caused by certain enzymes (Mustaffa, Osman, Yusof, & Mohamed, 1998; Tribess, 2009).

The results of moisture, soluble solids, titratable acidity and firmness after 14 days of storage are shown in Table 2 and compared with data obtained by Ditchfield and Tadini (2002). These authors evaluated the physicochemical characteristics of banana Nanicão (*Musa cavendishii* Lamb.) based on eight stages of maturation. Stage 1 corresponded to fruits with green color and stage 8 was reached when banana showed yellow peel with large brown spots. The values of soluble solids, titratable acidity and firmness presented in Table 2 by Ditchfield and Tadini (2002), corresponded to stage 8 of maturation. The evaluated soluble solids were similar to those found by these authors, as

Table 4
Electrical conductivity (σ) and dynamic viscosity (μ) of NADES diluted with different amounts of water at 25 °C.

NADES	Water content[a]									
	0	10	20	30	40	50	60	70	80	90
σ [mS·cm^{-1}]										
CA:BA	169.6 ± 2.3^{d}	1189.3 ± 8.8^{e}	3640.5 ± 10.2^{f}	7041.1 ± 15.2^{f}	10824.0 ± 29.9^{f}	13,679.0 ± 4.1^{f}	15,320.5 ± 29.9^{f}	15,241.4 ± 14.7^{f}	13,090.7 ± 8.5^{f}	8458.0 ± 5.1^{e}
CA:CC	118.1 ± 2.9^{e}	1959.3 ± 6.3^{d}	8543.3 ± 4.8^{d}	19,717.9 ± 10.1^{d}	31783.0 ± 9.7^{d}	41,457.4 ± 38.5^{d}	46,163.4 ± 7.6^{d}	45,891.0 ± 19.3^{d}	39,399.5 ± 22.9^{d}	25,881.9 ± 5.2^{c}
CA:Fru	0.2 ± 0.0f	41.5 ± 2.0^{f}	278.8 ± 28.2^{gh}	921.5 ± 19.5^{gh}	1941.8 ± 3.2^{g}	3161.8 ± 14.6^{g}	4305.5 ± 31.7^{g}	5175.6 ± 49.5^{g}	5404.5 ± 5.6^{g}	4594.6 ± 3.7^{f}
CA:Glu	0.2 ± 0.0^{f}	23.8 ± 2.3^{f}	214.1 ± 3.5^{gh}	772.3 ± 32.2^{gh}	1712.6 ± 22.2^{g}	2891.3 ± 17.4^{h}	4061.0 ± 11.8^{g}	4973.2 ± 34.6^{g}	5271.4 ± 8.4^{g}	4528.7 ± 0.4^{f}
Fru:CC	278.7 ± 23.3^{c}	3300.5 ± 169.2^{b}	11,650.5 ± 286.4^{b}	23,898.6 ± 801.8^{b}	36164.1 ± 752.7^{b}	46,860.6 ± 37.7^{b}	51,558.6 ± 357.4^{b}	50,761.1 ± 314.5^{b}	42,438.6 ± 81.2^{b}	26,350.6 ± 96.7^{b}
Glu:CC	198.2 ± 6.6^{d}	2740.9 ± 26.7c	10,591.1 ± 144.8^{c}	22,583.8 ± 198.1^{c}	35176.6 ± 4.3^{c}	45,226.9 ± 63.0^{c}	50,448.8 ± 168.7^{c}	49,700.7 ± 10.3^{c}	41,740.5 ± 271.2^{c}	26,369.5 ± 241.5^{b}
MA:BA	1021.5 ± 13.7^{b}	3444.2 ± 15.4^{b}	7282.3 ± 65.8^{e}	11,555.4 ± 5.5^{e}	15549.5 ± 27.2^{e}	18,429.6 ± 20.9^{e}	19,591.1 ± 14.0^{e}	18,743.7 ± 62.1^{e}	15,563.3 ± 39.3^{e}	9672.7 ± 22.9^{d}
MA:CC	1525.9 ± 13.2^{a}	7362.3 ± 52.0^{a}	18,499.2 ± 6.4^{a}	31,975.5 ± 17.7^{a}	44169.7 ± 13.0^{a}	52,570.8 ± 40.3^{a}	55,877.5 ± 32.9^{a}	53,047.8 ± 23.3^{a}	44,116.3 ± 67.2^{a}	28,397.0 ± 4.1^{a}
MA:Fru	12.3 ± 1.2^{f}	115.8 ± 0.1^{f}	434.0 ± 15.4^{g}	1010.1 ± 19.2^{g}	1762.1 ± 4.9^{g}	2607.4 ± 8.9^{h}	3366.0 ± 22.5^{h}	3893.3 ± 12.5^{h}	3986.3 ± 34.3^{h}	3350.1 ± 31.2^{g}
MA:Glu	8.5 ± 0.2^{f}	95.3 ± 1.2^{f}	375.1 ± 7.3^{gh}	896.7 ± 3.4^{gh}	1600.5 ± 9.8^{g}	2419.2 ± 8.2^{i}	3185.6 ± 17.8^{h}	3728.0 ± 20.9^{h}	3854.5 ± 7.5^{h}	3276.0 ± 3.2^{g}
U:Fru	0.8 ± 0.1^{f}	6.9 ± 1.0^{f}	21.8 ± 2.5^{h}	44.4 ± 4.3^{h}	70.4 ± 7.9^{h}	89.3 ± 5.6^{j}	102.9 ± 1.6^{i}	101.1 ± 7.7^{i}	85.4 ± 3.6^{i}	51.6 ± 1.1^{h}
U:Glu	0.4 ± 0.1^{f}	4.5 ± 0.1^{f}	16.1 ± 0.3^{h}	33.5 ± 1.6^{h}	58.2 ± 3.4^{h}	75.1 ± 8.0^{j}	84.1 ± 6.1^{i}	83.6 ± 9.4^{i}	72.6 ± 11.6^{i}	47.7 ± 11.9^{h}
μ [mPa·s^{-1}]										
CA:BA	7082.0 ± 52.2^{c}	409.9 ± 2.4^{c}	76.9 ± 3.8^{c}	26.2 ± 1.5^{b}	10.0 ± 0.8^{abcd}	5.8 ± 0.4^{a}	3.2 ± 0.0^{abc}	2.1 ± 0.1^{abc}	2.1 ± 0.1^{a}	1.4 ± 0.1^{ab}
CA:CC	4080.8 ± 17.4^{c}	178.3 ± 1.0^{ef}	32.7 ± 0.4^{f}	14.0 ± 0.7^{c}	5.7 ± 0.8^{ef}	3.3 ± 0.4^{bcd}	2.3 ± 0.1^{cd}	1.4 ± 0.0^{c}	1.2 ± 0.1^{a}	0.9 ± 0.1^{c}
CA:Fru	115,039.0 ± 1562.7^{b}	1384.8 ± 15.2^{b}	125.1 ± 1.9^{b}	28.4 ± 0.3^{b}	12.3 ± 1.2^{ab}	5.8 ± 0.8^{a}	3.1 ± 0.5^{abc}	2.3 ± 0.0^{abc}	2.0 ± 0.5^{a}	1.2 ± 0.0^{bc}
CA:Glu	437,768.5 ± 10,308.9^{a}	3006.2 ± 42.0^{a}	187.1 ± 1.6^{a}	37.9 ± 1.9^{a}	12.9 ± 0.1^{a}	5.9 ± 0.1^{a}	3.4 ± 0.0^{ab}	2.1 ± 0.3^{abc}	1.6 ± 0.0^{a}	1.7 ± 0.0^{a}
Fru:CC	2504.4 ± 67.6^{c}	183.1 ± 5.7^{ef}	37.8 ± 0.3^{ef}	15.0 ± 0.6^{c}	7.0 ± 0.7^{def}	3.1 ± 0.1^{cd}	2.3 ± 0.1^{cd}	1.9 ± 0.0^{abc}	1.6 ± 0.0^{a}	1.1 ± 0.1^{bc}
Glu:CC	3939.4 ± 104.3^{c}	229.2 ± 11.2^{de}	42.3 ± 0.0^{de}	14.7 ± 0.1^{c}	9.0 ± 0.7^{bcde}	5.6 ± 0.5^{a}	3.4 ± 0.0^{ab}	2.6 ± 0.1^{ab}	1.4 ± 0.0^{a}	1.0 ± 0.0^{c}
MA:BA	615.3 ± 14.0^{c}	113.6 ± 6.4^{g}	37.2 ± 5.5^{ef}	15.0 ± 0.6^{c}	9.2 ± 0.7^{abcde}	4.9 ± 0.6^{abc}	3.2 ± 0.2^{abc}	2.0 ± 0.0^{abc}	1.2 ± 0.1^{a}	0.9 ± 0.1^{c}
MA:CC	304.4 ± 6.9^{c}	56.3 ± 3.7^{h}	20.5 ± 2.2^{g}	8.8 ± 1.4^{d}	4.9 ± 0.5^{f}	2.4 ± 0.4^{d}	2.1 ± 0.0^{d}	1.8 ± 0.1^{bc}	1.4 ± 0.1^{a}	0.9 ± 0.1^{c}
MA:Fru	2343.1 ± 138.9^{c}	207.9 ± 11.8^{def}	44.8 ± 1.8^{de}	16.3 ± 0.6^{c}	8.5 ± 0.5^{cdef}	5.5 ± 1.1^{ab}	3.5 ± 0.5^{a}	3.0 ± 0.7^{a}	2.1 ± 0.7^{a}	1.1 ± 0.2^{bc}
MA:Glu	3735.3 ± 2.6^{c}	242.9 ± 5.1^{d}	48.4 ± 1.7^{d}	17.4 ± 0.7^{c}	12.0 ± 2.3^{abc}	5.0 ± 0.1^{abc}	3.2 ± 0.3^{abc}	2.2 ± 0.4^{abc}	1.5 ± 0.1^{a}	1.3 ± 0.2^{bc}
U:Fru	2048.8 ± 2.1^{c}	154.8 ± 0.7^{fg}	40.6 ± 1.8^{def}	16.3 ± 1.7^{c}	7.3 ± 0.4^{def}	5.2 ± 0.6^{abc}	2.8 ± 0.1^{abcd}	2.1 ± 0.2^{abc}	1.4 ± 0.1^{a}	1.1 ± 0.1^{bc}
U:Glu	2638.1 ± 42.5^{c}	176.4 ± 3.5^{ef}	37.2 ± 0.3^{ef}	14.6 ± 1.8^{c}	7.1 ± 0.6^{ef}	4.1 ± 0.5^{abcd}	2.6 ± 0.1^{bcd}	1.8 ± 0.3^{bc}	1.5 ± 0.1^{a}	1.0 ± 0.0^{bc}

All data are expressed as the mean ± SD of duplicate samples. Different letters in the same column indicate that there is significant difference ($P \le 0.05$).

[a] Water content = amount of water added to NADES (g/100 g).

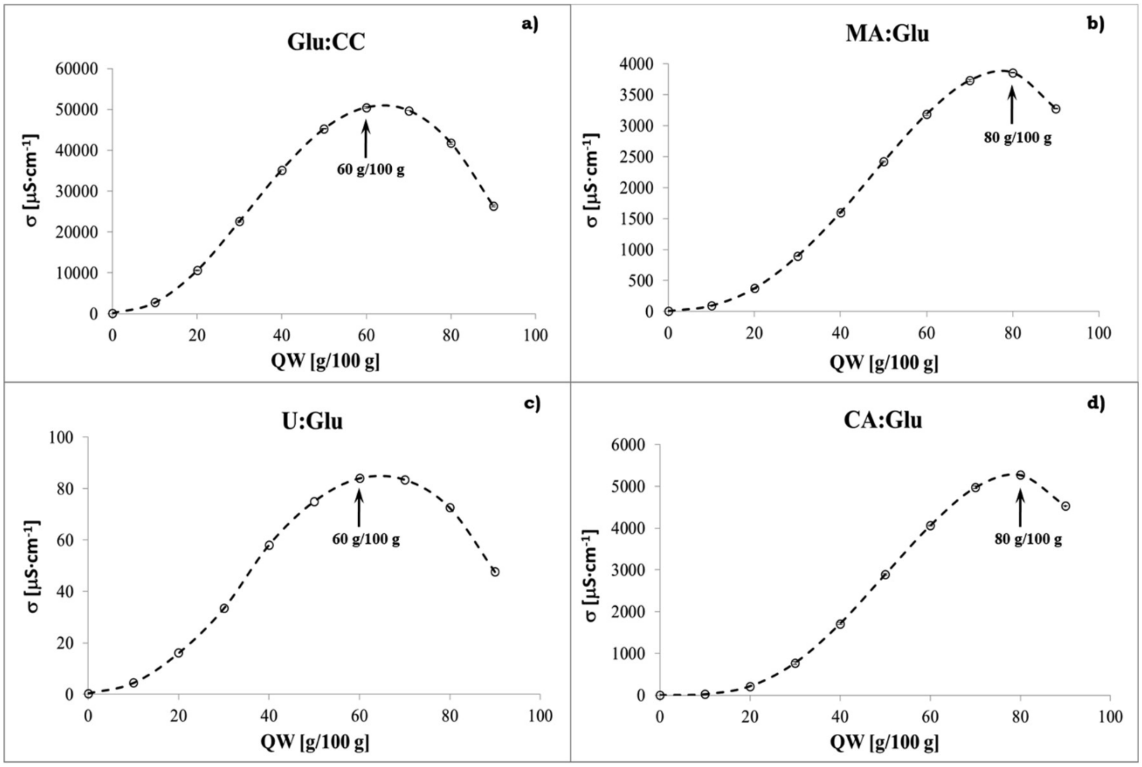

Fig. 1. Electrical conductivity (σ) versus quantity of water added to NADES (QW). **a)** Glu:CC = glucose:choline chloride, **b)** MA:Glu = malic acid:glucose, **c)** U:Glu = urea:glucose, **d)** CA:Glu = citric acid:glucose. Arrows indicate the highest conductivity values. The same tendencies were found in all the others NADES samples.

well as titratable acidity. After reviewing several assays, Ditchfield and Tadini (2002) concluded that the best method to estimate the degree of maturation of bananas was to measure the firmness using a texture analyzer. According to the firmness value obtained in this work (0.801 N) and in comparison with that shown in Table 2 (0.804 N), it was concluded that the bananas used in this study were in stage 8 of maturation.

3.2. Characterization of NADES

In view of previous reports (Dai et al., 2015; Pena-Pereira, Kloskowski, & Namiesnik, 2015), several NADES were prepared and tested by combining different compounds at different molar ratios, times, and melting temperatures (Table 3).

NADES prepared with tartaric, glutamic, and oxalic acids at several molar ratios were not capable of forming a clear liquid solution. Based on the best combinations of molar ratio, temperature and time, twelve mixtures were selected to measure their physicochemical properties (Table 3). Although lactic acid was able to form a clear liquid, it was discarded because of its high cost. Using lactic acid to prepare NADES would result in a high cost of production.

The high viscosity of NADES hinders the efficiency of them as extraction solvents due to the low mass transport efficiency. In order to remove this hindrance and improve the viscosity, several dilutions of NADES (0–90 g/100 g) were prepared and their physicochemical properties were measured.

3.2.1. Electrical conductivity

Without the addition of water, NADES prepared with malic acid, choline chloride and b-alanine (MA:CC, MA:BA) presented the highest conductivities (1525.9 and 1021.5 µS cm^{-1}, respectively), while NADES with citric acid or malic acid:sugar (CA:Glu, CA:Fru, MA:Fru, MA:Glu) and urea:sugar (U:Glu, U:Fru) showed the lowest values of conductivity (Table 4). The conductivity of NADES decreased in the following sequence: MA:CC > MA:BA » > Fru:CC > Glu:CC ≅ CA:BA > CA:CC » MA:Fru ≅ MA:Glu ≅ U:Fru ≅ U:Glu ≅ CA:Glu ≅ CA:Fru. This tendency could be attributed to the ionic form of choline chloride and the partial ionization of malic acid (pK_a = 3.13), citric acid (pK_a = 3.46), b-alanine (pK_a = 3.63) and urea (pK_a = 0.2). Thus, it may be concluded that NADES with malic acid and choline chloride showed the highest conductivity while NADES prepared with urea had low values.

Conductivity of NADES diluted with different amounts of water (0–90 g/100 g) first increased with increasing water content, and then decreased after reaching a peak value of around 10–100 times higher than that of pure NADES (Table 4). Samples prepared with malic acid:sugar (MA:Glu, MA:Fru) and citric acid:sugar (CA:Glu, CA:Fru) had the highest conductivity at 80 g/100 g of water addition, while NADES containing choline chloride (Glu:CC, Fru:CC, MA:CC, CA:CC), urea (U:Glu, U:Fru) and b-alanine (MA:BA, CA:BA) reached the highest value with 60 g/100 g of water dilution (Fig. 1). Therefore, the conductivity of all NADES increased as a result of the decrease in viscosity, due to the dilution caused by specific amounts of water. Consequently, electrical conductivity of NADES, as well as viscosity, could be tailored by changing the water content.

3.2.2. Water activity

NADES prepared with b-alanine (MA:BA, CA:BA) showed the highest values of water activity (0.653 and 0.629, respectively), while NADES with sugar:choline chloride (Glu:CC, Fru:CC) showed the lowest values (0.180 and 0.177, respectively) (Table 5). The water activity of NADES decreased in the following sequence: MA:BA > CA:BA > MA:Fru ≅ MA:Glu > U:Fru ≅ U:Glu > CA:CC ≅ CA:Fru > MA:CC > CA:Glu > Glu:CC ≅ Fru:CC. The incorporation of water to NADES caused a gradual increase in the water activity. With 60 g/100 g of water incorporated, all samples reach a water activity higher than 0.900.

Table 5
Water activity (a_w) at 25 °C, refractive index (RI) at 20 °C and density (ρ) at 40 °C of NADES diluted with different amounts of water.

NADES	Water content[a]									
	0	10	20	30	40	50	60	70	80	90
a_w										
CA:BA	0.629 ± 0.00^{b}	0.755 ± 0.01^{a}	0.843 ± 0.00^{a}	0.892 ± 0.00^{a}	0.928 ± 0.01^{a}	0.945 ± 0.00^{a}	0.958 ± 0.00^{a}	0.974 ± 0.00^{a}	0.983 ± 0.00^{ab}	0.989 ± 0.00^{a}
CA:CC	0.409 ± 0.00^{e}	0.584 ± 0.00^{d}	0.714 ± 0.00^{e}	0.796 ± 0.00^{d}	0.851 ± 0.00^{e}	0.897 ± 0.00^{cd}	0.931 ± 0.00^{cd}	0.957 ± 0.01^{abc}	0.975 ± 0.01^{abc}	0.987 ± 0.00^{a}
CA:Fru	0.409 ± 0.01^{e}	0.640 ± 0.01^{c}	0.764 ± 0.00^{bcd}	0.847 ± 0.00^{b}	0.893 ± 0.01^{bc}	0.935 ± 0.00^{a}	0.956 ± 0.00^{a}	0.974 ± 0.00^{a}	0.982 ± 0.00^{ab}	0.994 ± 0.00^{a}
CA:Glu	0.366 ± 0.01^{g}	0.587 ± 0.01^{d}	0.728 ± 0.01^{e}	0.831 ± 0.01^{bc}	0.888 ± 0.01^{cd}	0.931 ± 0.01^{ab}	0.957 ± 0.01^{a}	0.974 ± 0.01^{a}	0.986 ± 0.01^{a}	0.996 ± 0.01^{a}
Fru:CC	0.177 ± 0.00^{h}	0.367 ± 0.00^{f}	0.542 ± 0.00^{g}	0.676 ± 0.00^{f}	0.781 ± 0.00^{g}	0.855 ± 0.01^{e}	0.906 ± 0.00^{e}	0.942 ± 0.00^{c}	0.968 ± 0.00^{bc}	0.984 ± 0.00^{a}
Glu:CC	0.180 ± 0.00^{h}	0.380 ± 0.01^{f}	0.552 ± 0.01^{g}	0.683 ± 0.00^{f}	0.785 ± 0.00^{g}	0.857 ± 0.01^{e}	0.907 ± 0.01^{e}	0.945 ± 0.01^{c}	0.964 ± 0.00^{c}	0.986 ± 0.00^{a}
MA:BA	0.653 ± 0.00^{a}	0.759 ± 0.00^{a}	0.831 ± 0.01^{a}	0.875 ± 0.01^{a}	0.909 ± 0.00^{ab}	0.929 ± 0.01^{ab}	0.947 ± 0.00^{ab}	0.967 ± 0.00^{ab}	0.980 ± 0.01^{abc}	0.985 ± 0.01^{a}
MA:CC	0.387 ± 0.00^{f}	0.558 ± 0.01^{e}	0.675 ± 0.00^{f}	0.765 ± 0.00^{e}	0.828 ± 0.00^{f}	0.874 ± 0.01^{de}	0.916 ± 0.00^{de}	0.945 ± 0.00^{c}	0.968 ± 0.00^{bc}	0.983 ± 0.00^{a}
MA:Fru	0.518 ± 0.00^{c}	0.666 ± 0.01^{b}	0.776 ± 0.00^{b}	0.838 ± 0.00^{bc}	0.886 ± 0.00^{cd}	0.926 ± 0.01^{ab}	0.949 ± 0.00^{ab}	0.969 ± 0.01^{ab}	0.981 ± 0.00^{abc}	0.993 ± 0.01^{a}
MA:Glu	0.510 ± 0.01^{c}	0.667 ± 0.01^{b}	0.767 ± 0.01^{bc}	0.836 ± 0.00^{bc}	0.886 ± 0.01^{cd}	0.923 ± 0.01^{ab}	0.949 ± 0.00^{ab}	0.967 ± 0.00^{ab}	0.982 ± 0.00^{ab}	0.991 ± 0.00^{a}
U:Fru	0.483 ± 0.00^{d}	0.650 ± 0.00^{bc}	0.753 ± 0.00^{cd}	0.825 ± 0.01^{c}	0.874 ± 0.00^{cd}	0.909 ± 0.00^{bc}	0.936 ± 0.00^{bc}	0.957 ± 0.00^{abc}	0.977 ± 0.01^{abc}	0.990 ± 0.00^{a}
U:Glu	0.481 ± 0.00^{d}	0.643 ± 0.00^{bc}	0.749 ± 0.00^{d}	0.826 ± 0.01^{c}	0.872 ± 0.01^{d}	0.908 ± 0.00^{bc}	0.938 ± 0.00^{bc}	0.953 ± 0.01^{bc}	0.977 ± 0.00^{abc}	0.993 ± 0.01^{a}
RI										
CA:BA	1.486 ± 0.00^{e}	1.469 ± 0.00^{d}	1.451 ± 0.00^{c}	1.435 ± 0.00^{d}	1.418 ± 0.00^{c}	1.402 ± 0.00^{c}	1.387 ± 0.00^{c}	1.372 ± 0.00^{bc}	1.359 ± 0.00^{cd}	1.345 ± 0.00^{bc}
CA:CC	1.482 ± 0.00^{f}	1.466 ± 0.00^{e}	1.449 ± 0.00^{c}	1.432 ± 0.00^{d}	1.417 ± 0.00^{c}	1.401 ± 0.00^{c}	1.387 ± 0.00^{bc}	1.371 ± 0.00^{cd}	1.359 ± 0.00^{cd}	1.345 ± 0.00^{bc}
CA:Fru	1.494 ± 0.00^{d}	1.474 ± 0.00^{c}	1.455 ± 0.00^{b}	1.437 ± 0.00^{c}	1.419 ± 0.00^{c}	1.402 ± 0.00^{c}	1.387 ± 0.00^{c}	1.373 ± 0.00^{bc}	1.359 ± 0.00^{cd}	1.345 ± 0.00^{bc}
CA:Glu	1.499 ± 0.00^{bc}	1.477 ± 0.00^{b}	1.458 ± 0.00^{b}	1.440 ± 0.00^{b}	1.422 ± 0.00^{b}	1.405 ± 0.00^{b}	1.389 ± 0.00^{b}	1.374 ± 0.00^{b}	1.359 ± 0.00^{bc}	1.346 ± 0.00^{ab}
Fru:CC	1.502 ± 0.00^{ab}	1.482 ± 0.00^{a}	1.463 ± 0.00^{a}	1.444 ± 0.00^{a}	1.427 ± 0.00^{a}	1.409 ± 0.00^{a}	1.392 ± 0.00^{a}	1.377 ± 0.00^{a}	1.362 ± 0.00^{ab}	1.347 ± 0.00^{a}
Glu:CC	1.503 ± 0.00^{a}	1.483 ± 0.00^{a}	1.464 ± 0.00^{a}	1.445 ± 0.00^{a}	1.427 ± 0.00^{a}	1.409 ± 0.00^{a}	1.392 ± 0.00^{a}	1.377 ± 0.00^{a}	1.362 ± 0.00^{a}	1.347 ± 0.00^{a}
MA:BA	1.473 ± 0.00^{g}	1.458 ± 0.00^{g}	1.442 ± 0.00^{ef}	1.426 ± 0.00^{g}	1.411 ± 0.00^{e}	1.397 ± 0.00^{d}	1.383 ± 0.00^{d}	1.370 ± 0.00^{cd}	1.357 ± 0.00^{e}	1.345 ± 0.00^{bc}
MA:CC	1.473 ± 0.00^{g}	1.457 ± 0.00^{g}	1.441 ± 0.00^{f}	1.426 ± 0.00^{fg}	1.411 ± 0.00^{e}	1.397 ± 0.00^{d}	1.383 ± 0.00^{d}	1.370 ± 0.00^{cd}	1.357 ± 0.00^{de}	1.345 ± 0.00^{c}
MA:Fru	1.480 ± 0.00^{f}	1.462 ± 0.00^{f}	1.445 ± 0.00^{de}	1.428 ± 0.00^{ef}	1.413 ± 0.00^{de}	1.397 ± 0.00^{d}	1.383 ± 0.00^{d}	1.370 ± 0.00^{d}	1.357 ± 0.00^{de}	1.345 ± 0.00^{c}
MA:Glu	1.483 ± 0.00^{f}	1.464 ± 0.00^{ef}	1.446 ± 0.00^{d}	1.429 ± 0.00^{e}	1.413 ± 0.00^{d}	1.398 ± 0.00^{d}	1.384 ± 0.00^{d}	1.370 ± 0.00^{d}	1.357 ± 0.00^{de}	1.345 ± 0.00^{bc}
U:Fru	1.497 ± 0.00^{cd}	1.477 ± 0.00^{b}	1.457 ± 0.00^{b}	1.439 ± 0.00^{bc}	1.421 ± 0.00^{b}	1.405 ± 0.00^{b}	1.388 ± 0.00^{b}	1.374 ± 0.00^{b}	1.359 ± 0.00^{abc}	1.346 ± 0.00^{ab}
U:Glu	1.498 ± 0.00^{cd}	1.477 ± 0.00^{b}	1.458 ± 0.00^{b}	1.439 ± 0.00^{bc}	1.422 ± 0.00^{b}	1.405 ± 0.00^{b}	1.389 ± 0.00^{b}	1.374 ± 0.00^{b}	1.360 ± 0.00^{abc}	1.346 ± 0.00^{ab}
ρ [g·cm^{-3}]										
CA:BA	1.394 ± 0.01^{d}	1.352 ± 0.00^{c}	1.304 ± 0.00^{d}	1.259 ± 0.00^{c}	1.215 ± 0.00^{e}	1.175 ± 0.00^{c}	1.135 ± 0.00^{c}	1.097 ± 0.00^{ab}	1.060 ± 0.00^{c}	1.026 ± 0.00^{c}
CA:CC	1.289 ± 0.00^{g}	1.255 ± 0.00^{f}	1.223 ± 0.00^{g}	1.191 ± 0.00^{f}	1.160 ± 0.00^{i}	1.129 ± 0.00^{g}	1.100 ± 0.00^{f}	1.071 ± 0.00^{bcde}	1.044 ± 0.00^{f}	1.018 ± 0.00^{g}
CA:Fru	1.428 ± 0.00^{b}	1.394 ± 0.01^{b}	1.337 ± 0.01^{b}	1.288 ± 0.00^{b}	1.238 ± 0.00^{b}	1.190 ± 0.00^{b}	1.146 ± 0.00^{b}	1.087 ± 0.03^{abc}	1.065 ± 0.00^{b}	1.027 ± 0.00^{b}
CA:Glu	1.442 ± 0.00^{a}	1.407 ± 0.01^{a}	1.352 ± 0.00^{a}	1.295 ± 0.00^{a}	1.246 ± 0.00^{a}	1.197 ± 0.00^{a}	1.152 ± 0.00^{a}	1.108 ± 0.00^{a}	1.067 ± 0.00^{a}	1.029 ± 0.00^{a}
Fru:CC	1.250 ± 0.00^{h}	1.220 ± 0.00^{g}	1.192 ± 0.00^{h}	1.164 ± 0.00^{g}	1.142 ± 0.00^{j}	1.112 ± 0.00^{h}	1.087 ± 0.00^{g}	1.062 ± 0.00^{cde}	1.038 ± 0.00^{g}	1.015 ± 0.00^{h}
Glu:CC	1.246 ± 0.00^{h}	1.218 ± 0.00^{g}	1.192 ± 0.00^{h}	1.163 ± 0.00^{g}	1.138 ± 0.00^{k}	1.111 ± 0.00^{h}	1.085 ± 0.00^{h}	1.060 ± 0.00^{de}	1.037 ± 0.00^{g}	1.014 ± 0.00^{h}
MA:BA	1.340 ± 0.00^{f}	1.301 ± 0.00^{e}	1.263 ± 0.00^{f}	1.224 ± 0.00^{e}	1.189 ± 0.00^{h}	1.152 ± 0.00^{f}	1.117 ± 0.00^{e}	1.085 ± 0.00^{abcd}	1.052 ± 0.00^{e}	1.022 ± 0.00^{f}
MA:CC	1.229 ± 0.00^{i}	1.204 ± 0.00^{h}	1.179 ± 0.00^{i}	1.154 ± 0.00^{h}	1.129 ± 0.00^{l}	1.105 ± 0.00^{i}	1.081 ± 0.00^{i}	1.058 ± 0.00^{e}	1.035 ± 0.00^{h}	1.014 ± 0.00^{i}
MA:Fru	1.396 ± 0.00^{d}	1.356 ± 0.00^{c}	1.310 ± 0.00^{c}	1.260 ± 0.00^{c}	1.219 ± 0.00^{c}	1.175 ± 0.00^{c}	1.135 ± 0.00^{c}	1.097 ± 0.00^{ab}	1.060 ± 0.00^{c}	1.025 ± 0.00^{d}
MA:Glu	1.406 ± 0.01^{c}	1.354 ± 0.00^{c}	1.307 ± 0.00^{cd}	1.259 ± 0.00^{c}	1.217 ± 0.00^{d}	1.175 ± 0.00^{c}	1.134 ± 0.00^{c}	1.096 ± 0.00^{ab}	1.059 ± 0.00^{c}	1.025 ± 0.00^{d}
U:Fru	1.381 ± 0.00^{e}	1.334 ± 0.00^{d}	1.289 ± 0.00^{e}	1.246 ± 0.00^{d}	1.205 ± 0.00^{f}	1.165 ± 0.00^{d}	1.128 ± 0.00^{d}	1.092 ± 0.00^{ab}	1.056 ± 0.00^{d}	1.023 ± 0.00^{e}
U:Glu	1.378 ± 0.00^{e}	1.329 ± 0.00^{d}	1.284 ± 0.00^{e}	1.243 ± 0.00^{d}	1.203 ± 0.00^{g}	1.162 ± 0.00^{e}	1.128 ± 0.00^{d}	1.090 ± 0.00^{ab}	1.056 ± 0.00^{d}	1.023 ± 0.00^{e}

All data are expressed as the mean ± SD of duplicate samples. Different letters in the same column indicate that there is significant difference ($P \leq 0.05$).
[a] Water content = amount of water added to NADES (g/100 g).

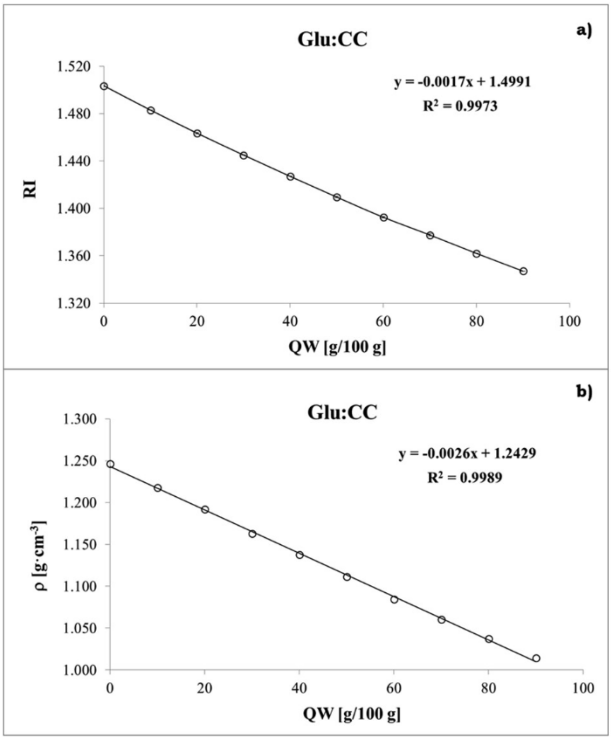

Fig. 2. Refractive index (RI) **(a)** and density (ρ) **(b)** curves (y = − ax + b) for NADES containing glucose and choline chloride (Glu:CC). QW: quantity of water added to NADES. The same trends were found in all the others NADES solvents.

Table 6
3^3 full factorial design and soluble sugars values obtained from banana puree using microwave-assisted extraction (MAE).

Run	Design matrix			Responses			
	Coded Factors			Soluble sugars (g/100 g) (dry base)			
	Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	$Y_{(CA:BA)}$	$Y_{(CA:CC)}$	$Y_{(MA:BA)}$	$Y_{(MA:CC)}$
1	−1	−1	0	77.3	87.5	65.6	77.0
2	−1	1	0	80.0	92.0	94.5	77.4
3	1	−1	0	73.1	85.0	65.1	76.8
4	1	1	0	73.7	89.3	70.2	79.0
5	0	−1	−1	71.7	71.6	46.4	72.4
6	0	−1	1	79.6	82.9	63.1	76.3
7	0	1	−1	75.5	77.0	59.7	76.0
8	0	1	1	86.1	85.7	67.5	79.3
9	−1	0	−1	74.3	82.5	98.8	72.2
10	1	0	−1	68.3	77.4	62.5	73.9
11	−1	0	1	82.3	89.6	65.5	75.8
12	1	0	1	78.4	86.3	68.3	75.8
13	0	0	0	69.7	81.4	66.4	78.9
14	−1	−1	−1	72.1	81.6	83.1	71.8
15	1	−1	−1	57.7	75.5	57.7	71.5
16	−1	1	−1	77.4	85.3	106.9	74.0
17	1	1	−1	68.0	87.9	64.8	86.6
18	−1	−1	1	82.1	88.0	43.8	72.6
19	1	−1	1	77.0	85.5	67.3	75.2
20	−1	1	1	85.1	101.5	95.7	76.2
21	1	1	1	80.2	87.2	71.2	85.5
22	−1	0	0	76.8	90.7	76.8	77.0
23	1	0	0	73.4	86.4	66.7	78.7
24	0	−1	0	66.8	78.0	61.7	75.9
25	0	1	0	77.9	83.6	67.3	82.6
26	0	0	−1	74.6	76.4	59.6	74.1
27	0	0	1	82.0	85.5	65.6	77.0

3.2.3. Soluble solids

The lowest refractive index values were found for NADES prepared with malic acid:b-alanine (MA:BA) and malic acid:choline chloride (MA:CC), and highest values were obtained for samples with sugar:choline chloride (Glu:CC and Fru:CC) when no dilutions were made to the NADES (Table 5). The refractive index decreased as the water content increased following the linear function y = -ax + b (y: refractive index, x: water content) (Fig. 2a). In all cases the value of R^2 was higher than 0.995. The fact that the refractive index is higher for samples with less quantity of water can be explained by the presence of molecules that are more polarizable than water. Therefore, the larger the number of polarizable molecules in the sample, the larger was the value of the refractive index.

3.2.4. Dynamic viscosity

Without the addition of water, citric acid:glucose sample (CA:Glu) showed the highest viscosity (437,768.5 mPa s^{-1}), followed by citric acid:fructose sample (CA:Fru) (115,039.0 mPa s^{-1}). There were no significant differences between the other NADES studied (Table 4).

The viscosity decreased significantly as the water content increased, and all NADES attained similar viscosity values when water content reached 70 g/100 g (Table 4). The high viscosity of NADES is often attributed to the presence of extensive hydrogen-bonding interactions between the components. Dilution with water leads to a large decrease in the viscosity of NADES as a result of the gradually weakened hydrogen-bonding interactions between the components.

3.2.5. Density

The densities of all tested NADES turned out to be higher than that of water. The highest densities were found for NADES prepared with citric acid:sugar (CA:Glu = 1.442 g cm^{-3}, CA:Fru = 1.428 g cm^{-3}). Lowest values were obtained in malic acid:choline chloride (MA:CC = 1.229 g cm^{-3}) and sugar:choline chloride samples (Glu:CC = 1.246 g cm^{-3}, Fru:CC = 1.250 g cm^{-3}) (Table 5). The density of NADES decreased in the following sequence: CA:Glu > CA:Fru > MA:Glu > CA:BA ≅ MA:Flu > U:Fru ≅ U:Glu > MA:BA > CA:CC > Glu:CC ≅ Fru:CC > MA:CC (Table 5).

As well as in the case of refractive index and viscosity, the density decreased with increasing water content. Densities of all tested NADES decreased linearly with the increasing water content following the function, y = -ax + b (y: density, x: water content) (Fig. 2b). In all samples, the value of R^2 was higher than 0.993.

3.3. Operational variables affecting soluble sugars (SS) extraction by MAE

Based on preliminary assays, NADES containing glucose and fructose were discarded because the high content of these monosaccharides on NADES hamper the SS determination. Therefore, four NADES (CA:BA, CA:CC, MA:BA, MA:CC) were selected to extract SS from banana puree. Table 6 shows the quantity of SS obtained from each experiment according to the factorial design.

Analysis of variance (ANOVA) was performed for the estimation of quantitative effects of the factors. Table 7 shows the effects of the quadratic model and associated *p* values for the response. All the operational variables studied affected the extraction process, showing significant effects and/or interactions for the evaluated response. When MA:BA was used as the extraction solvent, the temperature (X_1) presented a significant effect ($p < 0.05$) of negative signal, which revealed that the SS extraction was enhanced when using a temperature of 25 °C. The time (X_2) had a positive significant effect on SS extraction ($p < 0.05$), which suggested that the extraction percentage was improved when the time was increased. The interaction between temperature and time (X_1X_2) also showed a highly significant effect ($p < 0.05$) with a negative signal. Although the amount of water added to NADES (X_3) did not show a significant effect on SS extraction, the interaction between temperature and water content (X_1X_3) was significant ($p < 0.05$) with a positive signal. Quadratic temperature term

Table 7
The quantitative factor effects and associated *p*-value for the responses $Y_{(CA:BA)}$, $Y_{(CA:CC)}$, $Y_{(MA:BA)}$ and $Y_{(MA:CC)}$. X_1 (Temperature), X_2 (time), X_3 (quantity of water added to NADES): main effects. X_1X_2, X_1X_3 and X_2X_3: interaction terms. X_1^2, X_2^2, X_3^2: polynomial terms.

Factor	$Y_{(CA:BA)}$		$Y_{(CA:CC)}$		$Y_{(MA:BA)}$		$Y_{(MA:CC)}$	
	Factor effect	*P* Value	Factor effect	*P* Value	Factor effect	*P* Value	Factor effect	*P* Value
X_1	−6.3704	0.0007*	−4.2867	0.0010*	−16.2630	0.0000*	3.4658	0.0045*
X_2	5.1502	0.0041*	6.0123	0.0000*	16.6041	0.0000*	5.0980	0.0001*
X_3	10.2001	0.0000*	8.5868	0.0000*	−3.9776	0.2068	2.3196	0.0451*
X_1X_2	0.4436	0.8202	−0.6333	0.6451	−15.1120	0.0006*	3.6542	0.0114*
X_1X_3	2.5438	0.2045	−1.8455	0.1908	18.2840	0.0001*	−0.2926	0.8269
X_2X_3	−0.9189	0.6393	−0.5570	0.6858	2.9934	0.4272	−0.7017	0.5994
X_1^2	0.3634	0.8906	12.2938	0.0000*	20.6365	0.0005*	−1.6483	0.3646
X_2^2	1.4618	0.5940	0.0513	0.9787	−5.6615	0.2858	0.0008	0.9997
X_3^2	6.1367	0.0270*	−4.6530	0.0181*	−3.7262	0.4575	−5.5859	0.0046*

*$P \leq 0.05$.

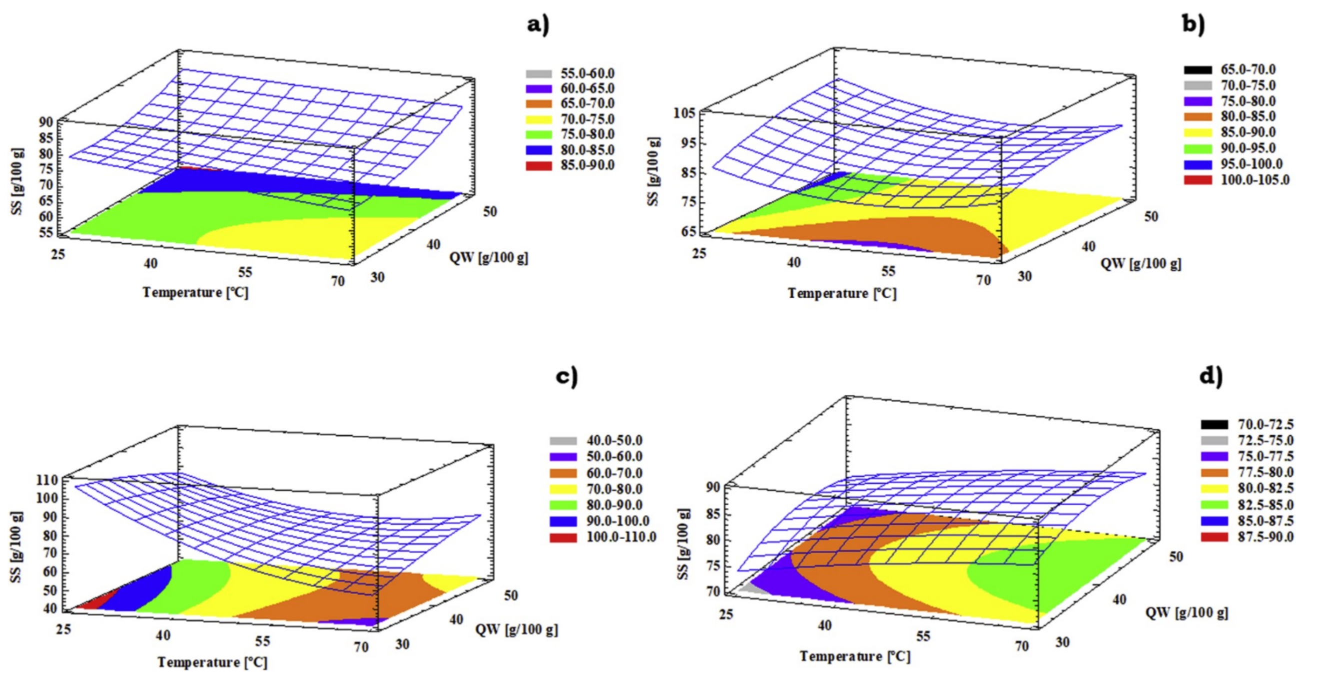

Fig. 3. Response surfaces for soluble sugars (SS) as a function of temperature and quantity of water added to NADES (QW) (based on the second-order polynomial equations): **a)** CA:BA, **b)** CA:CC, **c)** MA:BA, **d)** MA:CC.

Table 8
Optimum conditions, predicted and experimental values of responses ($Y_{(CA:BA)}$, $Y_{(CA:CC)}$, $Y_{(MA:BA)}$, $Y_{(MA:CC)}$) under those conditions.

	$Y_{(CA:BA)}$	$Y_{(CA:CC)}$	$Y_{(MA:BA)}$	$Y_{(MA:CC)}$
Predicted value*	85.7	96.5	106.3	84.7
Experimental value*	86.1	101.5	106.9	86.6
Relative error [%]	−0.4	−5.2	−0.6	−2.3
Temperature [°C]	25	25	25	70
Time [min]	30	30	30	30
QW** [g/100 g]	50	50	30	41.2

*Soluble sugars (g/100 g, dry base), **QW = quantity of water added to NADES.

X_1^2 also showed a significant, positive effect ($p < 0.05$) (Table 7). In the case of MA:CC solvent, all main effects (X_1, X_2, X_3) presented a significant positive effect ($p < 0.05$). Interaction term X_1X_2 and quadratic factor X_3^2 also showed a significant effect with values of $p = 0.0114$ and $p = 0.0046$, respectively (Table 7). The results for the SS extracted with CA:BA showed that all the main factors presented a significant effect ($p < 0.05$), while the only quadratic term that had a significant effect was X_3^2. The same tendency was found when the SS were extracted with CA:CC solvent. In this last case the term X_1^2 also presented a significant effect ($p < 0.05$) on the extraction of SS (Table 7).

A multiple regression analysis was then performed to fit the second-order polynomial equations to the experimental data points. The quantity of SS (g/100 g) extracted with the four selected NADES (CA:BA, CA:CC MA:BA, MA:CC) was correlated as a function of temperature (X_1), time (X_2) and QW (X_3), resulting in Eqs. (1)–(4), respectively.

$$Y_{(CA:BA)} = 115.7260–0.4156X_1 + 0.1518X_2 – 2.1489X_3 + 0.0008 \times 1 \times _2 + 0.0056 \times 1 \times _3 - 0.0038 \times 2 \times _3 + 0.0004 \times _1^2 + 0.0048 \times _2^2 + 0.0307 \times _3^2 \ (R^2 = 0.7997) \quad (1)$$

$$Y_{(CA:CC)} = 45.2862–1.0650X_1 + 0.3773X_2 + 2.5243X_3 – 0.0011 \times 1 \times _2 – 0.0041 \times 1 \times _3 – 0.0022 \times 2 \times _3 + 0.0121 \times _1^2 + 0.0002 \times _2^2 – 0.0233 \times _3^2 \ (R^2 = 0.8875) \quad (2)$$

$$Y_{(MA:BA)} = 154.3790–3.4525X_1 + 2.0954X_2 – 0.8479X_3 – 0.0269 \times 1 \times _2 + 0.0406 \times 1 \times _3 + 0.0120 \times 2 \times _3 + 0.0204 \times _1^2 – 0.0181 \times _2^2 – 0.0186 \times _3^2 \ (R^2 = 0.8534) \quad (3)$$

$$Y_{(MA:CC)} = 21.3295 + 0.1440X_1 + 0.0075X_2 + 2.4304X_3 + 0.0065 \times 1 \times {}_2 - 0.0006 \times 1 \times {}_3 - 0.0028 \times 2 \times {}_3 - 0.0016 \times {}_1^2 + 0.2443E^{-5}X_2^2 - 0.0279 \times {}_3^2 \ (R^2 = 0.7434) \quad (4)$$

Correlation coefficients (R^2) values were between 0.7434 and 0.8875, which showed a close agreement between experimental results and the theoretical values predicted by the second-order polynomials.

Three-dimensional plots described by the above-mentioned second-order polynomials were well fitted to the experimental data points through response surface graphs, confirming the suitability of the proposed quadratic models to explain the response variations in the range of values studied. Fig. 3 represents the variations in SS extraction (g/100 g) according to the evaluated variables. As can be seen, the surfaces clearly indicate a region where the value of the response is maximized. On the basis of the observed and predicted values and the quadratic model equations developed, the formulations were optimized. The optimal factors for the response (Y_i) are shown in Table 8. It can be seen that in all cases there was a good agreement between the predicted and the experimental values, since low values of relative error were found. The optimum extraction time was 30 min for all NADES. The optimum quantity of water added to NADES (QW) was 50 g/100 g for solvents containing citric acid (CA:BA, CA:CC). In the case of MA:BA and MA:CC, the best QW was 30 g/100 g and 41.18 g/100 g, respectively. The optimum temperature was 25 °C when MA:BA, CA:BA and CA:CC were used as extraction solvents, while for SS extracted with MA:CC was 70 °C (Table 8). The highest quantity of SS (106.9 g/100 g) was obtained with MA:BA solvent at 25 °C, 30 min and QW 30 g/100 g. On the basis of the conditions mentioned above, SS were extracted from banana puree with ethanol (99.5 mL/100 mL) and water during 30 min. The quantity of SS extracted with ethanol and water at 25 °C was 79.7 and 71.5 g/100 g, respectively; and at 70 °C was 88.7 g/100 g and 81.9 g/100 g, respectively. In all cases, the quantity of SS extracted with NADES were higher than those obtained with ethanol and water.

4. Conclusions

This study demonstrates that judicious mixtures of several compounds can form natural deep eutectic solvents (NADES) at optimal ratios at a certain temperature range. Despite high viscosity, all NADES evaluated were liquids at room temperature. The physicochemical properties can be tailored in a controllable way when diluted with small amounts of water.

In view of our physicochemical characterization studies, four NADES (CA:BA, CA:CC, MA:BA, MA:CC) were chosen for the extraction of soluble sugars (SS) from banana puree. Microwave-assisted extraction (MAE) was studied under various conditions to analyze the effects of temperature, time and quantity of water added to NADES on the extraction of SS from banana puree. A 3^3 full factorial design was successful in the evaluation of the significance of the three relevant reaction parameters. In all cases, the quantity of SS extracted increased with increasing time. Through optimization of reaction conditions, the highest quantity of SS (106.9 g/100 g) was obtained with MA:BA solvent at 25 °C, 30 min and a QW of 30 g/100 g. When this result was compared with the extraction made with ethanol (99.5 mL/100 mL) and water, the use of NADES was clearly more effective than these two traditional extraction solvents. Therefore, MA:BA was selected as the most promising solvent and proved to be effective in the extraction of SS from banana puree compared to conventional solvents. In view of these results, the use of NADES in the extraction process could represent highly efficient and truly eco-friendly extraction methods to replace current harsh organic solvents like ethanol.

This work shows the potential of using NADES as environmentally friendly solvents to extract soluble sugars from fruits like bananas. Further studies will be needed to achieve a better understanding of the complex nature of NADES and their interactions with food compounds.

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