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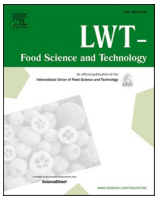

Microwave-assisted extraction (MAE) of bioactive compounds from blueberry by-products using a sugar-based NADES: A novelty in green chemistry

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ABSTRACT

The development of new green solvents is one of the key issues in Green Chemistry. In this context, a three component NADES based on Glucose:Glycerol:Lactic Acid (molar ratio of 1:2:5) was used to prepare a ready-to-use extracts, suitable for several application The Design of the Experiment (DoE) approach was used to optimize microwave assisted extraction (MAE) of anthocyanins from blueberry by-products. Four parameters were evaluated: extraction heat (EH), extraction time (ET), ramp/isotherm ratio (R/I) and solvent/matrix ratio (S/M). The D-optimal model was applied and validated by analysis of variance and "lake of fit" test. Finally using the optimized extraction conditions (EH: 60 °C; ET: 30 min; R/I: 2 min/min and S/M: 20 mL/g), the extraction efficiency of the sugar-based NADES was compared with extractions performed with a choline chloride-based NADES and an organic solvent (Methanol:HCl). The new solvent studied showed a high extraction performance, better than the conventional solvents used.

1. Introduction

Food production and consumption generate large amounts of biomass waste from processing, which poses a serious disposal problem. The agricultural sector contributed 4.4% ($85.1 billion) to global Gross Domestic Product (GDP) in 2020 («World Development Indicators | The World Bank», s.d.). This reflects the economic importance of the sector and the need to reduce consumption-related waste, as the amount of municipal waste associated with food is increasing. It should also be noted that a large part of the total fruit production is processed into juice and wine. This processing results in a large amount of waste given by the processing industries. For example, about 20–30% of the fruit is wasted in blueberry juice production (Liu et al., 2021).

In a circular economy approach, it is crucial to recover the waste produced, given the high value of the by-products due to the presence of active compounds. Bioactive compounds derived from plants and fruits have attracted considerable research interest due to their health benefits.

The conventional methods for extracting phytochemicals are based on the use of organic solvents such as a mixture of water and ethanol, methanol or acetone (Lima et al., 2019). However, organic solvents are toxic and require treatments in order to remove the solvent before the bioactive compounds can be used (Joshi & Adhikari, 2019) Therefore, the development of new ecological extraction alternatives has been continuously pursued over the last decades (Torres-Valenzuela, Ballesteros-Gómez, & Rubio, 2020).

For this reason, the use of alternative solvents has become of interest in the context of the development of "green chemistry". Recently, a new green extraction technology known as natural deep eutectic solvent (NADES) has emerged to meet the requirements of biodegradability, sustainability, low toxicity, low cost and ease of production (Cunha & Fernandes, 2018). NADES are formed by complexing an acceptor (HBA), such as quaternary ammonium, with a hydrogen bond donor (HBD), such as urea, carboxylic acids or amines. NADES are composed of natural components, usually primary plant metabolites (e.g. sugars). Analyzing the chemical nature of their components, NADES can be classified into four categories: derivatives from organic acids, derivatives of choline chloride, mixtures of sugars and other combinations. Compared to other solvents, they have the advantage of emulating the natural solution pathway of water-insoluble primary and secondary metabolites in plants (Benvenutti, Zielinski, & Ferreira, 2019). Several studies have evaluated the use of NADES as green solvents for the

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recovery of bioactive compounds. In particular, extraction has been performed with choline chloride based NADES, which is the first NADES studied by Verpoorte et al. (Choi et al., 2011). Binary NADES composed of choline chloride: oxalic acid seems to be an effective solvent for the extraction of bioactive compounds from blueberry (Santos-Martín et al., 2023). NADES obtained by combining choline chloride and a sugar also show high extraction efficiency (Zannou & Koca, 2022).

Thus, all choline-based NADES have a good extraction performance. However, considering a possible application of the extract as an active agent, the use of choline chloride is limited. For example, choline esters and salts are prohibited in cosmetic formulations ("European Parliament and Council Regulation (EC) No 1223/2009 of 30 November 2009 on cosmetic products").

For these reasons it may be interesting to study different types of sugar-based NADES. Furthermore, there are example in the literature of their efficiency: NADES composed of fructose: lactic acid or sucrose: lactic acid showed a higher extraction efficiency of polyphenols in curcumin (Doldolova et al., 2021a), while NADES composed of lactic acid:sucrose and citric acid:glucose performed higher extraction capability for the extraction of phenolic compounds from coffee beans. Finally, lactic acid:glucose NADES was used as a vehicle for the extraction of bioactive compounds from plant materials (Espino et al., 2019).

Despite the good extraction capability of sugar-based NADES, there are few works highlighting the extraction capabilities of such solvents towards fruits as matrix.

Therefore, in order to realize a ready-to-use extract, it could be interesting to evaluate the extraction efficiency of sugar-based NADES on the extraction of bioactive compounds from fruit wastes. To improve the extraction efficiency, new extraction techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) or ultrasonic-assisted extraction (UAE) (Osorio-Tobón, 2020) can be used.

Regarding MAE, process parameters such as temperature, extraction time, solvent:matrix, microwave power affect the extraction yield of the process. Therefore, optimal operating conditions need to be defined for optimized applications of MAE.

Design of Experiments (DoE) has been widely used as a mathematical modelling approach to identify an efficient set of parameters (x1, x2, …, xn − 1, xn) and regression analysis has been used to identify polynomial equations that adequately fit experimental data. The DOE technique provides a useful tool for constructing surrogate models, such as response surface (RS) models, which incorporate the interaction effect between the responses and the factors. These surrogate models can be applied to analyze the responses and perform multi-objective optimization design (MOD) (Nunes, Alvarenga, de Souza Sant'Ana, Santos, & Granato, 2015; Putnik, Kovačević, Penić, Fegeš, & Dragović-Uzelac, 2016). Consequently, response surface methodology (RSM) includes statistical and mathematical techniques, that are useful for process development, improvement and optimization (Montgomery, 2017; Nunes et al., 2015). The D-optimal design is reported in the literature to be commonly used for product formulation in the food industry. Among numerous methods, the D-optimal method was chosen because it reduces the number of runs required to apply RSM with good quality, so D-optimal is more popular than the general-optimal design because it lessens the amount of resources and time (Ciğeroğlu et al., 2018).

In view of the above, the aim of this study is to optimize the Microwave Assisted Extraction of bioactive compounds from blueberry by-products using a three-component sugar-based NADES. Based on the results obtained by Silva et al. using a three-component NADES (choline: chloride:glycerol:citric acid) on blueberry, we studied a new formulation without choline chloride (Silva et al., 2020). The NADES studied is composed of glucose:lactic acid:glycerol ((Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013). Optimized extraction conditions were found using a Design of Experiments (DoE) approach to improve the recovery of anthocyanins content by RSM with D-optimal design. Specifically, the influence of four operating parameters (Extraction Heat, Extraction Time, Solvent/Matrix ratio, Heating Ramp/Isoterm ratio) on the extraction yield (antochyanins content) was evaluated.

Finally, the choline-based NADES studied by Silva et al., and an organic solvent (methanol) were chosen as a reference to compare the extraction efficiency of the sugar-based ones.

2. Materials and method

2.1. Chemicals

α-D-Glucose (96%), acid lactic solution (80%), glycerol, methanol, hydrochloric acid (HCl), choline chloride (ChCl), Folin–Ciocalteu reagent, acid gallic as total phenol content standard were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Raw materials

Blueberry by-products (mainly blueberry peels) from a juice production company near Torino, Italy, were used as extraction matrices and stored at −20 °C until use. Prior to extraction, the samples were lyophilized (Lyovapor L-200, Buchi, Cornaredo, Italy) and ground to obtain a visually homogeneous powder.

2.3. Preparation of sugar-based NADES

After a thorough bibliographic search (Ahmad & Pertiwi, 2018; Doldolova et al., 2021b; Espino et al., 2019; Pavlić et al., 2022), it was decided to test a new three-component sugar-based NADES solvent (SN). This solvent was prepared by mixing glucose, glycerol and lactic acid (Glu:Gly:AL) in a molar ratio of 1:2:5. The three components were stirred at 80 °C for 20 min to obtain a homogeneous liquid. The NADES mixture was then diluted with 25% water (w/w) to reduce the viscosity. The physicochemical properties of the sugar-based NADES were evaluated: pH 2.4 and ρ 1.18 g/cm.

2.4. Optimization of the extraction condition (MAE)

Extraction was performed using sugar-based NADES (SN) as solvent. The samples were treated using a microwave extractor (Ethos UP, Milestone Connect, Bergamo, Italy) equipped with an SK-15 high-pressure digestion rotor to improve the extraction efficiency. The microwave extractor was also equipped with two magnetrons for a total of 1900 W and the magnetron frequency used was 2.45 GHz. The lyophilized sample (2 g), to which the correct volume of NADES was added, was placed in a glass vial and then in a Teflon high-pressure digestor. The instrument can control the temperature with a thermometer probe placed in a pilot reactor.

For all extractions the power of the microwave was set at 800 W. Different experimental parameters were tested: extraction heat (EH), extraction time (ET), solvent/matrix ratio (S/M), heating ramp/isotherm ratio (R/I). The range set for the four parameters tested is:

A. Extraction Heat (°C): 60-70
B. Extraction Time (min): 15-30
C. Solvent/Matrix ratio (mL/g): 10-20
D. Heating Ramp/Isoterm ratio (min/min): 0.5–2

The R/I ratio comes from the instrument setting that allows to specify:

1. The time required to reach the set temperature ($Ramp_{time}$)
2. The time the samples remain at the set temperature ($Isotherm_{time}$).

After MAE treatment, the resulting extract was centrifuged (centrifuge Hermle Z 380, Ghosheim, Germany) for 10 min at 3500 rpm and

Table 1
D-optimal design and experimental results.

Runs	A Extraction heat (°C)	B Extraction time (min)	C Solvent/ Matrix ratio (mL/ g)	D Ramp/ Isotherm ratio (min/ min)	Response TAC (mg/ 100 g)
N1	70	30	10	0.5	589
N2	65	22.5	15	1.25	484
N3	60	30	20	2	617
N4	70	30	20	0.5	435
N5	60	15	10	2	405
N6	70	15	20	0.5	518
N7	60	30	10	2	398
N8	65	22.5	15	1.25	431
N9	70	15	10	2	562
N10	60	15	20	0.5	408
N11	60	30	20	0.5	572
N12	70	15	20	2	572
N13	65	22.5	15	1.25	463
N14	70	30	10	2	545
N15	60	15	10	0.5	396
N16	70	15	15	0.5	500
N17	70	30	20	2	561
N18	60	30	10	0.5	389
N19	60	15	20	2	418
N20	60	22.5	10	0.5	456
N21	65	30	20	2	620
N22	60	30	10	1.25	400

then the liquid and solid fractions were separated with a sieve. The extracts were stored at 4 °C until analysis.

2.5. Design of response surface methodology

The design of experiments software (Stat-Ease 360) was used to optimize the MAE. After defining the factors and responses of interest, an appropriate design type was selected, and an experimental worksheet table was generated applying a D-optimal design. Then, a response surface model (RSM) was used.

The experimental design used in this work had as dependent variable the total anthocyanin content (TAC), expressed as $mg_{cyanidin\text{-}3\text{-}glucoside}/100g_{lyophilized\ matrix}$, and as independent variables the EH (A), the ET (B) the S/M (C), and the R/I (D).

The applied D-optimal design resulted in 22 experimental runs. The experiments were carried out in a randomized order. Table 1 shows the run order, the experimental design and the observed response for the four variables and the 22 experimental runs generated. The optimization procedure was performed after model refinement with the aim of defining the processing conditions for the highest concentration of anthocyanins in the extract.

2.6. Extraction using organic solvent (OS)

To evaluate the extraction performance of sugar-based NADES, the results obtained with the optimized extraction conditions were compared with those obtained with the organic solvent.

Extraction was performed according to the protocol of Savikin et al. 10 g of samples were weighed into a centrifuge tube after the addition of 25 mL of extraction solution (500 mL methanol, 24 mL water, and 1.4 mL HCl 37%). After 120 min in the dark, the extracts were manually homogenized for about 1 min and then centrifuged for 15 min at 3500 rpm (Hermle Z 380 centrifuge, Ghosheim, Germany). Extractions were performed in triplicate.

2.7. Extraction using choline chloride-based NADES (CN)

To evaluate the extraction performance of sugar-based NADES, the results obtained with the optimized extraction conditions were compared with those obtained using choline chloride-based NADES.

In order to compare the results with a similar NADES solvent, extraction was performed using the three-component NADES studied by Silva et al. (Silva et al., 2020). NADES consisted of Choline Chloride, Glycerol and Citric Acid (ChCl:Gly:AC) (CN) in a molar ratio of 0.5:2:0.5. This solvent was prepared by mixing the three components for 30 min at 80° and diluted with 25% water (w/w) (Silva et al., 2020).

The microwave-assisted extraction with choline chloride-based NADES was carried out using the optimized extraction conditions designed for the sugar-based NADES.

2.8. Determination of TAC and TPC on the extract

To compare the extraction performance of the three solvents (SN, CN and OS), the total phenolic content (TPC) and the total anthocyanin content (TAC) were evaluated.

2.8.1. Total anthocyanin content (TAC)

The anthocyanin content analysis followed the pH differential protocol (Roidoung, Dolan, & Siddiq, 2017). The extract (50 μL) was diluted separately with 5 mL each of pH 1 (potassium chloride 0.025 mol/L) and pH 4.5 (sodium acetate 0.4 mol/L) buffer solution. The absorbance values of the solution were determined spectrophotometrically at both λ 515 nm and λ 700 nm (V-550, Jasco).

The anthocyanin content was determined according to the following formula:

$$AC\left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

- AC: anthocyanin content as mg cyanidin-3-glucoside/l.
- A: difference of absorbances ((*A*520nm-*A*700nm) pH*1*-(*A*520nm-*A*700nm) pH*4.5*)
- MW: molecular weight of cyanidin-3-glucoside (499.384 g/mol)
- DF: dilution coefficient (10)
- l: optical path in cm
- ε: extinction coefficient (3040 L/mol*cm)

Results are expressed as mg/100 g dry fruit. Three replicates were performed for each treatment.

2.8.2. Total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu reagent with gallic acid as a standard (Slinkard & Singleton, 1977). The absorbance was measured at 760 nm. TPC was expressed as mg gallic acid equivalents (GAE) per 100 g dried weight. Three replicates were performed for each treatment.

2.9. Statistical analysis of the experimental results

The design of experiments, analysis of results, prediction of responses and statistical analyses were carried out using Stat-Ease 360 Software (Stat-Ease Inc, Minneapolis). ANOVA analysis was used to compare the extraction efficiency (TAC and TPC) of the three different solvents using Minitab 21.1 software (Minitab, LLC, Pennsylvania). Means were compared using Tukey's test ($p \leq 0.05$).

3. Results and discussion

3.1. Evaluation of regression model

The D-optimal design and its results for the extraction of anthocyanins from blueberry industrial waste using MAE are shown in Table 1.

Based on preliminary work, it was assumed that the individual factors and their interactions would be the most influential in determining the response (Alchera, Ginepro, & Giacalone, 2022). For this reason, the data were fitted to a modified Two Factor Interaction (2FI) regression

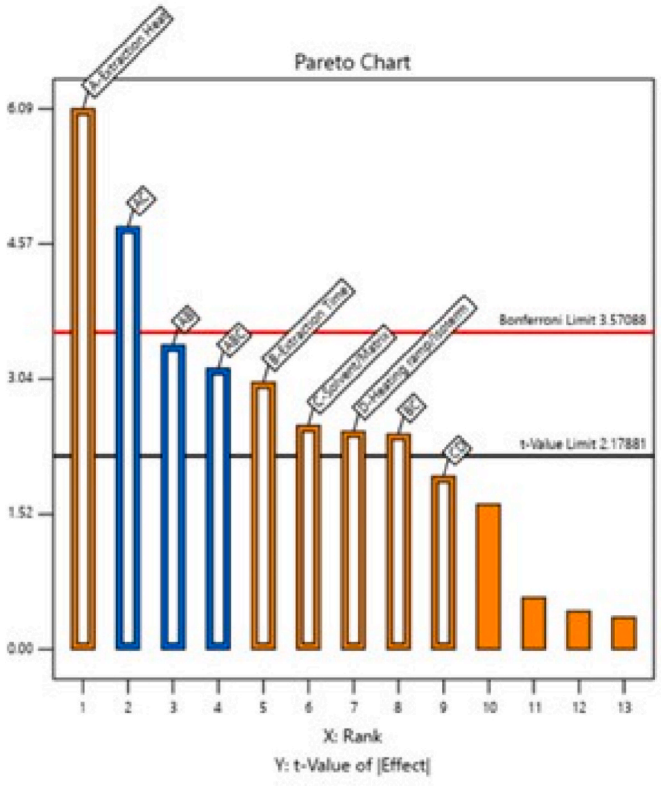

Fig. 1. Pareto chart for the TAC response. A: Extraction Heat; B: Extraction Time; C: Solvent/Matrix ratio; D: Heating ramp/Isoterm Orange column indicate positive effects; blue column indicates negative effects. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
ANOVA table for D-optimal design (response TAC).

Source	Sum of Sqaures	Df	Mean Square	F-value	p-value	
Block	2587.89	1	2587.89			
Model	1.258 E+05	9	13978.34	16.57	< **0.0001**	significant
A-Extraction Heat	31252.29	1	31252.29	37.05	**0.0001**	
B-Extraction Time	7609.31	1	7609.31	9.02	**0.0133**	
C-Solvent/ Matrix	5358.36	1	5358.36	6.35	**0.0304**	
D-Heating ramp/ Isotherm	5081.63	1	5081.63	6.02	**0.0340**	
AB	9909.60	1	9909.60	11.75	**0.0065**	
AC	19095.26	1	19095.26	22.64	**0.0008**	
BC	4953.75	1	4953.75	5.87	0.0591	
CD	3197.85	1	3197.85	3.79	0.0801	
ABC	8429.54	1	8429.54	9.99	**0.0069**	
Curvature	3261.10	1	3261.10	3.87	0.0776	
Residual	8435.31	10	843.53			
Lack of Fit	7010.65	8	876.33	1.23	0.5229	not significant
Pure Error	1424.67	2	712.33			
Cor Total	1.401 E+05	21				

model. The 2FI has the advantage of simultaneously considering the interaction between two factors and their effect on the response. By evaluating the Pareto chart (Fig. 1) the 2FI model was modified to consider the influence of the factors on the model.

The Pareto plot shows the absolute value of the effects from the largest to the smallest. For this reason, the interaction between the three factors ABC was included and the interactions and BD were excluded.

As result, a polynomial regression equation was developed. The equation for the fitted modified 2FI regression model for the TAC in coded factors is shown in Eq. (1).

$$Y = 496 + 43.51*A + 21.24*B + 18.31*C + 17.27*D - 25.04*AB - 36.15\ AC + 17.95*BC + 18.51*CD - 29.72*ABC \quad (1)$$

where, Y is the extraction yield of the TAC. In the regression equation, the negative sign assigned to the coefficient indicates an inverse effect between the factor and the response variable. Alternatively, a positive sign indicates a synergistic effect.

ANOVA was then used to analyze the significance of the model equation, the variables (factors) and their interaction.

The ANOVA table is reported in Table 2. The significant coefficients (in bold) were extraction heat (A), extraction time (B), Solvent/Matrix ratio (C), Heating Ramp/Isotherm ratio (D). The interactions AB, AC, ABC were also significant. In particular, the more significant coefficients (lower p-value) are extraction heat (A), extraction time (B) and their interaction (AB) and the interaction between extraction heat and solvent/matrix ratio (AC).

As reported in Table 3, the model was significant at the 95% confidence level (p-value <0.0001), and no lack of fit was observed (F-value of 1.23 and p-value of 0.5229). The Lack of Fit F-value of 1.23 implies that the model fits the data well. The R^2 value was also used to examine the goodness of fit of the responses. The closer the R^2 to 1, the better the model fits the data. An R^2 value of 0.9372 is very close to 1, indicating that the model has a good fit performance between the parameters and the response. The R^2 adj indicates what percentage of the variation in the dependent variable is explained by all the independent variables together. An R^2 adj. value of 0.9080 is in good agreement with the Pred R^2 (0.731), implying the model has a high predictive accuracy and a good consistency between the predicted values and the experimental values. This result is in line with the principle of ANOVA, as this analysis works by comparing the variation due to the treatment, with the variation due to the random errors inherited along with the dimensions of the generated responses. Additionally, the relatively low CV% (5.98%) ensures that the reliability between the actual and predicted values is much higher. Furthermore, the standard deviation of the model was as low as 29.04 for the anthocyanin content.

It is essential to analyze the adequacy of the mathematical model developed in order to prevent it from producing misleading or poor results. For this reason, an analysis was carried out on the residuals, which must have a normal distribution and be independent and identically distributed, i.e. with homogeneous variance. Therefore, the adequacy of the developed mathematical model was investigated by constructing analytical plots (Fig. 2). The normal distribution of the residuals, checked using Q-Q plot, is reported in Fig. 2A. Fig. 2B indicates the accuracy of prediction versus actual values for each experimental run. From these figures, it can be observed that the values were fitted around the regression line. Fig. 2C shows the relationship between the experimental runs and the residuals. The residuals (experimental errors obtained by subtracting the observed responses from the predicted responses) were randomly scattered in an irregular pattern, i.e., they were independent of the factors. This indicates the efficiency of the model. In addition, all the data points were within ±4, which is within the 95% confidence limits depending on the number of runs. This also indicates that no outliers were found when the data points were fitted. Finally, in the plots reported in Fig. 2D, the internally standardized residuals are plotted versus the predicted values. The randomly scattered data points confirm the homogeneous variance assumption.

Table 3
Summary table of ANOVA for TAC.

Response	Model type	F-value	p-value	Lack of fit	Standard deviation	C.V.%	R^2	AdjR^2	Predicted R^2
TAC	2FI	16.57	<0.0001	1.23	29.04	5.98	0.9372	0.9080	0.731

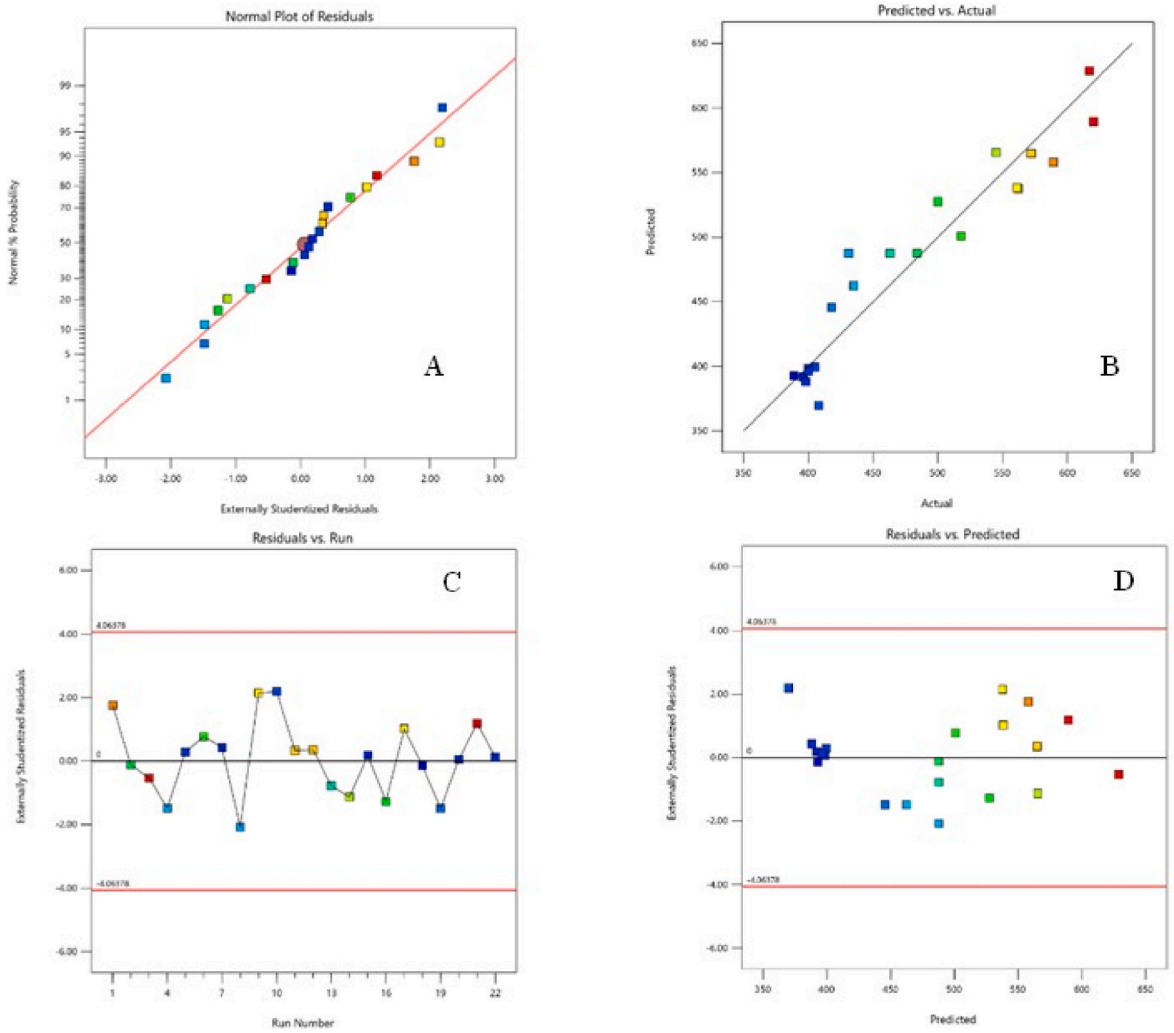

Fig. 2. Analytical plots to verify the appropriateness of the model for the TAC response. Fig A. Normal plot. Fig B. Predicted vs actual plot. Fig C. Residuals vs run plot. Fig D. Residuals vs predicted plot.

3.2. Optimization of anthocyanins extraction and model verification

A 3D curve analysis was carried on to evaluate the influence of operational parameters on the response (TAC). The peaks and valleys correspond to combinations of x and y that produce local maxima or minima. The analysis was performed based on the most significant factors obtained from the ANOVA analysis. Extraction heat (A) and extraction time (B) were found to be the two most influential parameters (without considering their interaction). Fig. 3 shows the 3D response curve, keeping the solvent/matrix ratio and the heating ramp/Isotherm constant.

Four different 3D response curves were analyzed. Comparing Fig. 3A and B and Fig. 3C and D, reducing the solvent-to-matrix ratio from 20 mL/g to 10 mL/g results in a change in EH. Using an S/M ratio of 20 mL/g results in higher TAC values with a lower EH (60 °C). Conversely, using 10 mL/g results in a higher extraction efficiency with a higher EH (70 °C). In both cases, better results are obtained with higher ET equal to 30 min.

The results could be related to solvent properties such as viscosity and density ($\rho = 1.18$ g/cm^2; $\mu = 29$ Cps). Very high viscosities make it difficult for the sample to interact with the NADES mixture, as extensive hydrogen bonding can limit the mobility of free species within the solvent, resulting in slow mass transfer (Zainal-Abidin, Hayyan, Hayyan, & Jayakumar, 2017). Considering the high viscosity of the NADES, using fewer solvent results in a significantly denser system (solvent + lyophilized matrix). In general, increasing the temperature produces a decrease in solvent viscosity and surface tension, resulting in greater wetting of samples and increased matrix penetration, which generally increases the solubility of antioxidant compounds (Bi, Tian, & Row, 2013). For these reasons, using less solvent a higher extraction temperature is required to reduce the mass transfer barrier and improve the extraction capacity. On the other hand, it has been reported that phenolics may decompose when high temperatures are reached, (Moldovan, Popa, & David, 2016; Zhu, Zhang, Tsang, Huang, & Chen, 1997).

Evaluating the 3D curves, an extraction conducted using a solvent/matrix ratio of 10 mL/g does not show a maximum point of the response

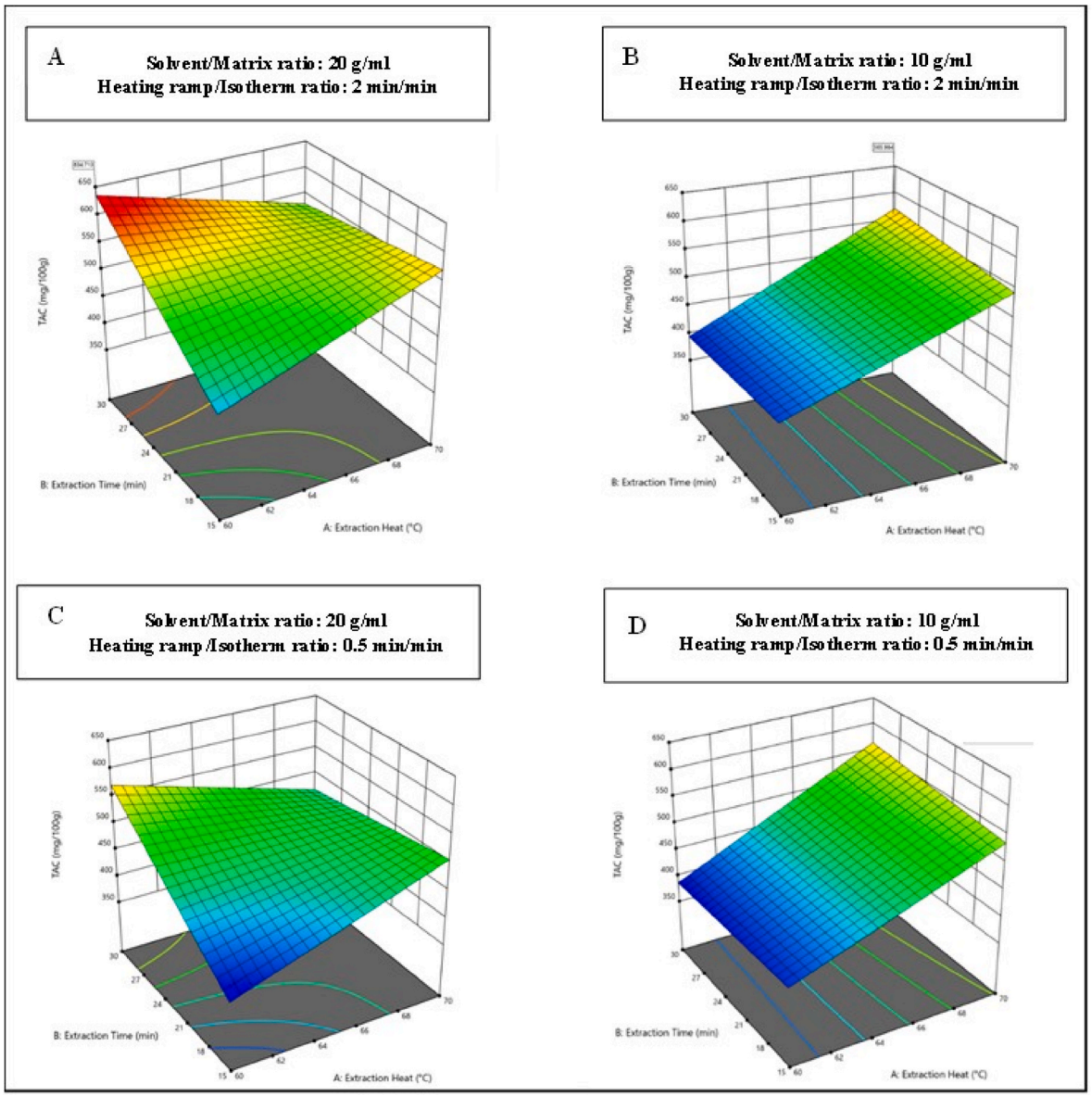

Fig. 3. 3D response curve for the TAC response: evaluation of different extraction conditions on the response.

surface plot. Indeed, Fig. 3B and D (solvent/matrix ratio set to 10 g/mL) show no curvature. This trend may be related to the high temperatures required for extraction under these conditions. In view of the above, it is preferable to work with higher solvent/matrix ratios and lower extraction temperatures to evaluate the conditions that lead to maximization of TAC yield.

Evaluating the influence of changing the heating ramp/isotherm ratio (comparing Fig. 3A vs 3C), no influence on the EH and ET parameters is observed. The R/I ratio was evaluated to test the thermal stability of the solvent. In fact, high temperature changes (faster ramp$_{time}$) could lead to solvent and matrix degradation and, consequently, lower extraction yield (Buckow, Kastell, Terefe, & Versteeg, 2010). A higher R/I value indicates slower heat-up (e.g., an R/I of 2 min/min for a total extraction time of 30 min means 20 min to reach the set temperature and 10 min at isotherm).

Using high or low R/I, changes the maximum reached by the 3D response curve, but the optimum extraction condition remains the same as analyzed above. Considering all the 3D response curve, in order to obtain higher TAC values, it is convenient to work with a higher R/I equal to 2 min/min. A higher R/I results in a slower heating of the system to the set extraction temperature.

Table 4
Optimized extraction conditions for the TAC response.

VARIABLE	OPTIMIZED CONDITION	UNIT
EXTRACTION HEAT (EH)	60	°C
EXTRACTION TIME (ET)	30	min
SOLVENT/MATRIX RATIO (S/M)	20	mL/g
HEATING RAMP/ISOTHERM (R/I)	2	min/min
TAC – TOTAL ANTHOCYANINS CONTENT	634.71	mg/100 g

Extraction efficiency of sugar-based NADES: comparison with organic solvent and Choline Chloride NADES.

Finally, Table 4 summarizes the combination of optimized parameters and the expected response obtained from the Design of Experiment. The optimal working conditions are the same found by analyzing the previous graphs. To verify the accuracy of the model, three extractions were performed using the optimized extraction conditions. The objective is to have a TAC similar to the results found by the DoE optimization

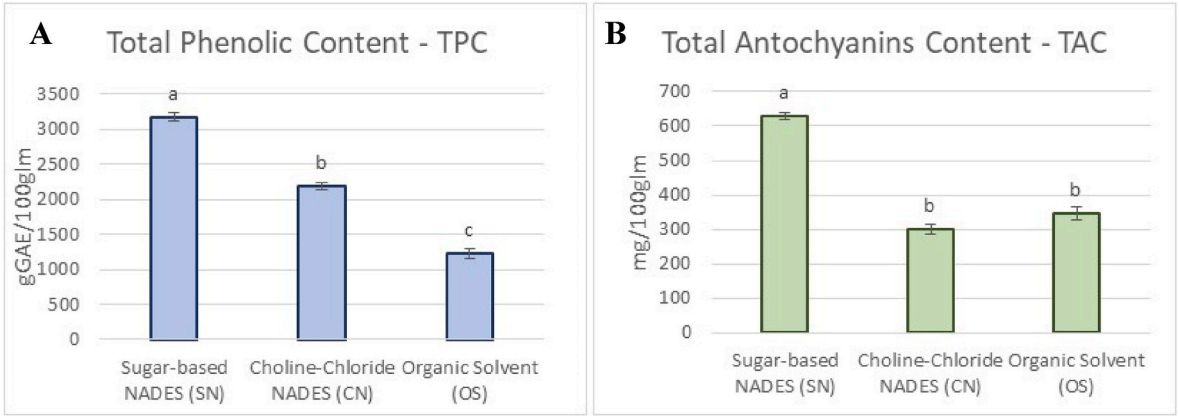

Fig. 4. Comparison of the extraction efficiency of sugar-based NADES vs organic solvent and choline chloride NADES. Graph A. total phenolic content (TPC); graph B total anthocyanins content (TAC).

software. The value obtained for the TAC was 628 ± 15.1 mg/100 g. The results were compared using one-way ANOVA test and no significant differences were found ($p > 0.05$). This confirms the adequacy of the studied model.

TPC was also evaluated in the extract obtained using the previously reported optimized extraction conditions. The results found for TPC and TAC were compared with those obtained for the extraction carried out with Choline-Chloride NADES (CN) and organic solvent (OS). The extraction performed with CN as solvent was carried out at the optimized extraction conditions found for the sugar-based NADES (SN). Three extractions were carried out for each solvent test. The results are reported in Fig. 4.

The results obtained for the CN solvent agree with those found in the literature. The study conducted by Silva et al. showed an anthocyanin concentration of 362.3 ± 1.1 mg/100 g lm, similar to our results (300 ± 2.2 mg/100 g lm) (Silva et al., 2020).

Instead, the results obtained with the organic solvent are consistent and slightly higher than those found in the literature (Hernández-Carrión & Narvaez, 2022; Muñoz-Fariña et al., 2023; Musilová et al., 2022; Silva et al., 2020). This value is probably related to the lyophilization process, that leads to a higher concentration of bioactive compounds and consequently to higher concentrations of TAC and TPC in the organic extracts.

Comparing the results obtained for the different types of extraction, the one conducted using sugar-based NADES results in a higher content of polyphenols and anthocyanins in the extract compared to the Choline Chloride NADES and the organic solvent.

The sugar-based NADES studied also shows better performance compared to other studies in the literature. Specifically, better performance is shown when compared to extractions using different types of NADES (Guo et al., 2019; Santos-Martín et al., 2023) and other types of natural solvents such as ethanol, water or citric acid (Routray & Orsat, 2014; Troncoso Mesa, Flórez-Méndez, López, & Bustos, 2021; Zannou & Koca, 2022).

4. Conclusion

Microwave-assisted extraction using NADES as solvent was found to be a suitable and effective method for the extraction of anthocyanins from blueberry by-products. The optimized extraction conditions were determined as follows: extraction heat (EH) of 60 °C, extraction time (ET) of 30 min, heating Ramp/Isotherm of 2 min/min and solvent/matrix ratio (S/M) of 20 mL/g. The sugar-based solvent tested proved to be an efficient extraction solvent with a higher extraction capacity than the organic one. The optimized extraction conditions resulted in a TAC of 634.71 mg/100 g of extract. Compared to conventional solvent extraction, the polyphenol yield was increased by 45% for TAC and 61% for TPC after application of the optimized MAE process. However, compared to choline chloride-based NADES, there was an increase of 52% for TAC and 31% for TPC.

In view of the results reported, sugar-based NADES could be considered as an interesting solvent for the extraction of bioactive compounds. It is a natural and non-toxic solvent that is suitable for use as an active agent in the cosmetic and packaging industries without the need for a solvent removal process.

Future research could investigate the correlation between the solvent viscosity and the extraction temperature. For this purpose, it could be interesting to modify the solvent viscosity by increasing the amount of water and to study the variation of optimized extraction conditions. Finally, considering the nature of the NADES components, the extracts obtained can be tested as antioxidant agents in cosmetic formulations. Regarding a cosmetic application, in order to reduce the acidity of the final extract, the influence of the amount of acid on the extraction yield could also be evaluated.

CRediT authorship contribution statement

Federica Alchera: Conceptualization, Formal analysis, Writing – original draft. **Marco Ginepro:** Formal analysis, Methodology. **Giovanna Giacalone:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Abbreviations

TAC	total anthocyanin content
TPC	total phenolic content
NADES	natural deep eutectic solvent
MAE	Microwave Assisted Extraction
EH	extraction heat
ET	extraction time
S/M	solvent/matrix ratio
R/I	heating ramp/isotherm ratio
CN	choline-Chloride NADES
OS	organic solvent

SN sugar-based NADES

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