Ultrasonics Sonochemistry 79 (2021) 105773

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Ultrasound-assisted extraction of phenolic acids, flavonols, and flavan-3-ols from muscadine grape skins and seeds using natural deep eutectic solvents and predictive modelling by artificial neural networking

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ARTICLE INFO

Keywords:
NDES
Extraction
Ultrasound
Ellagic
Grape
ANN

ABSTRACT

The objective of this study was to investigate the extraction efficiency of 9 natural deep eutectic solvents (NDES) with the assistance of ultrasound for phenolic acids, flavonols, and flavan-3-ols in muscadine grape (*Carlos*) skins and seeds in comparison to 75% ethanol. Artificial neural networking (ANN) was applied to optimize NDES water content, ultrasonication time, solid-to-solvent ratio, and extraction temperature to achieve the highest extraction yields for ellagic acid, catechin and epicatechin. A newly formulated NDES (#1) consists of choline chloride: levulinic acid: ethylene glycol 1:1:2 and 20% water extracted the highest amount of ellagic acid in the skin at 22.1 mg/g. This yield was 1.73-fold of that by 75% ethanol. A modified NDES (#3) consisting of choline chloride: proline: malic acid 1:1:1 and 30% water extracted the highest amount of catechin (0.61 mg/g) and epicatechin (0.89 mg/g) in the skin, and 2.77 mg/g and 0.37 mg/g in the seed, respectively. The optimal yield of ellagic acid in the skin using NDES #1 was 25.3 mg/g (observed) and 25.3 mg/g (predicted). The optimal yield of (catechin + epicatechin) in seed using NDES #3 was 9.8 mg/g (observed) and 9.6 mg/g (predicted). This study showed the high extraction efficiency of selected NDES for polyphenols under optimized conditions.

1. Introduction

Natural deep eutectic solvents (NDES) are prepared by mixing hydrogen-bond donors with hydrogen-bond acceptors at an appropriate molar ratio [1]. The melting point of one component should be lower than the melting point of the other one [1]. After heating and mixing, this medium becomes a liquid at room temperature. Water is added to stabilize and polarize the mixture. Research in the field of phytochemical extraction using NDES has expanded due to their effective extractability and solubility. Nonetheless, multiple factors play a significant role when comparing NDES to organic solvents, including yield, cost, recovery, and toxicity. Previous research investigated NDES on the extraction of different polyphenols from various food matrices. For example, Bubalo et al. (2016) compared 5 NDES, water, 70% methanol (v/v) and acidified 70% methanol (v/v) to extract anthocyanins, catechin and quercetin-3-*O*-glucoside from red grape skins. A NDES consisting of choline chloride: oxalic acid (1:1) with 25% of water (v/v) was found to be the most efficient extraction solvent [2]. In another study, Panić et al. (2019) tested 8 NDES and acidified 70% of ethanol and observed choline chloride: citric acid (2:1) with 30% water (v/v) as the best NDES to extract anthocyanins from grape pomace [3].

Muscadine grapes (*Vitis rotundifolia*) are native to the southeastern states and the first cultivated wild grape in the United States [4]. Muscadine grapes are produced in 12 states and total about 5000 acres [5]. There are 100 varieties of muscadine grape and each varies in physical, sensory, or chemical characteristics [4]. Among them, *Carlos* is a widely planted muscadine grape due to its high crop yields and growing consistency [4]. *Carlos* muscadine grape is medium in size, bronze in color, thicker in the skin and contains four seeds on average [6]. Muscadine grapes contain significant amounts of polyphenols which are known to reduce inflammation [7], inhibit prostate tumor growth [8] and improve metabolic responses of diabetics [9]. Muscadine grape pomace, a byproduct of muscadine grape juicing or winemaking, consists of skins and seeds. A previous research study used acetone:

Abbreviations: AAE, Average absolute error; ANN, Artificial neural networking; NDES, Natural deep eutectic solvents; RASE, Square root of the mean squared prediction error; SSE, Square and sum the prediction errors.

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https://doi.org/10.1016/j.ultsonch.2021.105773
Received 5 July 2021; Received in revised form 16 September 2021; Accepted 26 September 2021
Available online 8 October 2021

water: acetic acid mixture (70:29.7:0.3, v/v) to extract phenolic compounds from the seeds, skin, and pulp of eight cultivars of Florida-grown muscadine grape, including *Carlos* [10]. However, the use of flammable organic solvents and their low extraction efficiency hindered practical applications. Most of the muscadine grape pomace is still discarded as waste.

Artificial neural networking (ANN) is a nonlinear mapping system comprised of various basic processing units connected by weighted associations. These processing units are called "neurons" [11]. Artificial neural networking is a machine learning approach to predict or forecast a response based on multiple inputs [11]. Prior research has applied response surface methods (RSM) for extraction optimization and prediction. However, few studies have used ANN for the same purpose. For example, Sinha et al. (2013) suggested that ANN has better prediction performance than RSM on the extraction of natural dye from seeds of *Bixa orellana* (Annatto) [12]. In a similar study, Ciric et al. (2020) reported that ANN model was better than RSM for predicting phenolic compound extractions from garlic [13]. The objective of this research was to investigate the extraction efficiency of 9 NDES for phenolic acids, flavonols, and flavan-3-ols in comparison to 75% ethanol with the assistance of ultrasound. ANN was applied to predict and optimize the extraction conditions on phenolics yield. The hypothesis was that NDES with specific compositions extract higher amounts of phenolic acids, flavonols, and flavan-3-ols than 75% ethanol, and the highest extraction efficiency can be achieved by ANN-based predictive modelling.

2. Materials and Methods

2.1. Chemicals and reagents

Choline chloride, levulinic acid, 1,2-propanediol, DL-malic acid, oxalic acid, hydrochloric acid, and formic acid were obtained from Acros Organics (Morris Plains, NJ, USA). Lactic acid, ethylene glycol, glycine, HPLC-grade acetonitrile, methanol, and ethanol were purchased from Fishers Scientific (Waltham, Massachusetts, USA). L-proline and betaine hydrochloride were purchased from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade standards of ellagic acid, gallic acid, ferulic acid, (+)-catechin, (−)-epicatechin, myricetin, quercetin, and kaempferol were acquired from Sigma Aldrich (St. Louis, MO, USA).

Table 1
List of NDES used for the extraction of polyphenols from muscadine grape skin and seeds.

NDES	Composition	Molar Ratio	Water content (mL/100 mL)	pH	Reference
#1	Choline chloride: levulinic acid: ethylene glycol	1:1:2	20	2.27	[14]
#2	Choline chloride: 1,2-propanediol: lactic acid	1:1:2	10	1.04	[14]
#3	Choline chloride: proline: malic acid	1:1:1	30	3.01	Modified from [15]
#4	Choline chloride: betaine hydrochloride: ethylene glycol	1:1:2	20	1.74	[23]
#5	Choline chloride: 1,2-propanediol	1:1	10	3.33	[24]
#6	Proline: malic acid	1:1	32	2.80	[25]
#7	Lactic acid: glycine	4:1	20	2.74	[25]
#8	Choline chloride: lactic acid	1:2	20	0.60	[26]
#9	Choline chloride: oxalic acid	1:1	30	0.30	[2]

2.2. Design of NDES

NDES #1–2 in Table 1 were designed in our previous study [14]. Choline chloride in NDES #1–2 was selected as a hydrogen acceptor, whereas two different hydrogen donors were selected for each new NDES. Molar ratios between hydrogen donors and acceptor and water content were determined in preliminary experiments. NDES #3–9 in Table 1 were selected from the literature as previous studies have appointed them as effective NDES in extracting polyphenols. Water content in NDES #3 was modified from the cited literature. A heating method was applied to prepare the NDES [15]. Briefly, the hydrogen-bond acceptor was mixed with each of the hydrogen-bond donor components in Erlenmeyer flasks with a stirring bar. The mixture in the flask was closed and heated at 50 °C for about 30 min or until a clear liquid formed and remained stable at room temperature. Water contents in Table 1 were calculated according to the final volume of NDES mixtures. The pH of NDES listed in Table 1 was measured using a pH meter (AB15, Accumet, Fisher Scientific, Waltham, MA, USA).

2.3. Sample preparation / Ultrasound-assisted extraction

Frozen muscadine grape (*vitis rotundifolia*) skins and seeds (cultivar: *Carlos*) were provided by Paulk Vineyards (Wray, Georgia, USA). After removing hulls, leaves, or petioles, pomace was separated into seeds and skin. The samples were then dried using a vacuum oven (Isotemp, Model 285A, Fisher Scientific, Waltham, Massachusetts, USA) at 60 °C and a vacuum-pressure lower than −30 in.Hg. Next, the samples were homogenized into a fine powder using a chimerical grinder (A1000, RRH Inc., 2800 W, Zhejiang, China). Using an initial solid-to-solvent ratio of 1:20 (g:mL), 0.50 g of either muscadine grape skin or seed was mixed in 10 mL NDES or 75% ethanol in triplicates. The samples were then placed in a water-bath (60 °C) and sonicated (VCX 1500, Sonics & Materials Inc., 1500-Watt, 50/60 Hz, Newtown, CT, USA) for 30 min at 100% amplitude for two rounds (15 min/round). Next, the samples were immediately centrifuged (Sorvall ST 8, Fisher Scientific, Suzhou, China) at 3,260 *g* until a clear supernatant was obtained. Lastly, the supernatants were collected and stored in a −20 °C freezer for HPLC analysis of phenolic acids (ellagic acid, gallic acid, ferulic acid), flavonols (myricetin, quercetin, and kaempferol), and flavan-3-ols (catechin and epicatechin).

2.4. HPLC analyses of phenolic acids, flavonols, and flavan-3-ols

Phenolic acids, flavonols, and flavan-3-ols were analyzed on an HPLC system (Agilent Technologies 1200, Waldbronn, Germany) according to the method described in Sandhu and Gu (2013) [16]. The HPLC system consists of a binary pump, an autosampler, a thermostatted column compartment, a diode array detector and a fluorescence detector. Grape skin or seed extracts were hydrolyzed before the analyses of phenolic acids and flavonols. The hydrolysis was performed by mixing 1 mL of the extract with 4 mL of hydrolysis solution (1.2 M HCI contain 50% methanol) and placed in a water bath (Precision, Model 2837, 400 W, 50/60 Hz, Thermo Scientific, Marietta, OH, USA) at 90 °C for 80 min. Next, the samples were cooled down to 25 °C followed by sonication for 5 min. The hydrolysis of the extract was not needed for the analysis of catechin and epicatechin. The hydrolyzed and un-hydrolyzed extracts were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane before HPLC analyses. For analyzing ellagic acid, gallic acid, ferulic acid, myricetin, quercetin, kaempferol, catechin and epicatechin, 10 μL was injected into a SB-C18 column (4.6 × 250 mm, 5 μm, Zorbax, Agilent, Santa Clara, CA, USA). The mobile phases were (A) 0.5% formic acid and (B) 100% acetonitrile. The flow rate was 1 mL/min with 25 min modified gradient as follows: 0–5 min, 10–30% B; 5–10 min, 30–40% B; 10–20 min, 40–50% B; 20–25 min, 50–10% B; followed by 5 min of equilibration. The temperature of the column was set at 30 °C. The detection wavelength was 260 nm for ellagic acid, gallic acid and ferulic

acid and 360 nm for myricetin, quercetin and kaempferol on a photodiode array detector. The excitation and emission for catechin and epicatechin were 230 nm, 321 nm, respectively, using a fluorescence detector. Polyphenol compounds were quantified using standard curves of ellagic acid, gallic acid, ferulic acid, myricetin, quercetin, kaempferol, catechin and epicatechin. All standard curves had 7 points and $R^2 > 0.99$.

2.5. Customized design for artificial neural networking

Four independent extraction variables with four levels: water content (15–60%), ultrasonication time (5–35 min), solid-to-solvent ratio (1:5–1:20), and extraction temperature (30–60 °C) (Table S1) were applied to optimize the extraction yield of phenolic acids, flavonols, and flavan-3-ols. Unlike the classic designs such as the response surface design, ANN-based design does not require repeating runs and prefers a different data structure. In our previous study [14], ANN was a more reliable method for predicting extraction yield than RSM. Therefore, ANN was selected in this study to predict the extraction yield of ellagic acid, catechin and epicatechin. A customized design with 40 runs (Table S2) was generated on JMP Pro (Version 14.2, SAS Institute Inc., Cary, NC, USA) to provide data specifically for ANN predictive modeling. Randomization of the 40 runs was applied to eliminate any bias.

The main equation of ANN is shown as follows:

$$n_k^h = \sum_{j=1}^{j} w_{kj}^h p_j + b_k^h, k = 1toK \quad (1)$$

where h is the number of neurons in hidden layer, j *and* k are the number of input variables and hidden neurons, respectively, p is the input variable, b^h is the bias of the hidden layer, and w^h is the weight in the hidden layer.

Extraction yields of ellagic acid, catechin and epicatechin in relation to the four independent variables were analyzed using ANN by training the data first and then choosing the best activation type and number of neurons that results in an adequate fit of the data. To evaluate the success of prediction models, three values have been assessed: R-square, the square root of the mean squared prediction error (RASE) (equation (2)), and the average absolute error (AAE).

RASE is:

$$RASE = \sqrt{SSE/n} \quad (2)$$

Where *SSE* donates for square and sum the prediction errors (differences between the actual responses and the predicted responses) and n for number of observations. R-square close to 1 with RASE and AAE close to zero means a higher fit of the data into the model.

2.6. Statistics

Extraction yields of phenolic acids, flavonols, and flavan-3-ols were compared with one-way ANOVA followed by Student's t test at $p \leq 0.05$ using JMP Pro (Version 14.2, SAS Institute Inc., Cary, NC, USA). Each NDES and 75% ethanol were compared using Dunnett's tests at $p \leq 0.05$. Principle component analysis (PCA) was performed on JMP Pro (Version 14.2, SAS Institute Inc., Cary, NC, USA) for the phenolic compounds extracted from muscadine grape skin and seed.

3. Results and Discussion

3.1. Polyphenols extracted by NDES from muscadine grape skins

Nine NDES and 75% ethanol were utilized for the extraction of polyphenols from the muscadine grape skins. Table 2 shows the extraction yield of ellagic acid, gallic acid, ferulic acid, myricetin, quercetin, kaempferol, catechin and epicatechin. Ellagic acid was the most abundant extractable polyphenol in grape skin, followed by gallic acid and ferulic acid, respectively. This finding was consistent with previous studies [17,18].

NDES #1, #8, #7, #3, #2 and #9 extracted significantly higher amounts of ellagic acid in grape skin than 75% ethanol. The highest extraction yield of ellagic acid was achieved by NDES #1 followed by NDES #8 at 22.1 ± 2.2 mg/g and 21.3 ± 2.5 mg/g, respectively (Table 2). However, there was no significant difference between NDES #1 and NDES #8 according to Student's t test. Interestingly, NDES #1 was found to be the least effective NDES to extract anthocyanins from

Table 2
Extraction yield of phenolic acids, flavonols, and flavan-3-ols from grape skin by NDES and 75% ethanol.

NDES	Ellagic acid		Gallic acid		Ferulic acid		Catechin		Epicatechin		Myricetin		Quercetin		Kaempferol		Sum**
	mg/g*	p^	mg/g	p	mg/g	p	mg/g	p	mg/g	p	mg/g	p	mg/g	p	mg/g	p	mg/g
#1	22.1 ± 2.2 ^{a}	0.00	9.77 ± 1.1 ^{ab}	0.45	6.32 ± 0.7 ^{a}	0.76	0.05 ± 0.0 ^{def}	0.58	0.27 ± 0.0 ^{cd}	1.00	1.84 ± 0.2 ^{a}	0.89	0.40 ± 0.0 ^{ab}	1.00	0.04 ± 0.0 ^{ab}	0.55	**40.7**
#2	16.3 ± 2.1^{b}	0.04	8.38 ± 0.6^{c}	0.99	4.67 ± 0.3 ^{cd}	0.03	0.02 ± 0.0 ^{ef}	0.97	0.25 ± 0.0 ^{cd}	1.00	1.34 ± 0.1 ^{d}	0.01	0.34 ± 0.0 ^{ef}	0.00	0.04 ± 0.0 ^{bc}	0.14	**31.3**
#3	16.8 ± 0.2^{b}	0.02	8.93 ± 0.1 ^{bc}	1.00	5.38 ± 0.2 ^{bc}	0.73	0.61 ± 0.1 ^{a}	0.00	0.89 ± 0.1 ^{a}	0.00	1.46 ± 0.0 ^{cd}	0.11	0.35 ± 0.0 ^{def}	0.01	0.04 ± 0.0 ^{abc}	0.37	**34.4**
#4	7.44 ± 0.6 ^{e}	0.00	9.71 ± 0.1 ^{ab}	0.52	5.42 ± 0.1 ^{bc}	0.80	0.10 ± 0.0 ^{cd}	0.04	0.30 ± 0.0 ^{cd}	1.00	1.59 ± 0.0 ^{bc}	0.71	0.36 ± 0.0 ^{cde}	0.07	0.04 ± 0.0 ^{ab}	0.62	**24.9**
#5	8.42 ± 0.5^{de}	0.01	5.55 ± 0.1 ^{d}	0.00	3.11 ± 0.0^{f}	0.00	0.09 ± 0.0 ^{de}	0.08	0.14 ± 0.0 ^{d}	0.56	0.87 ± 0.0 ^{e}	0.00	0.27 ± 0.0 ^{g}	0.00	0.03 ± 0.0^{c}	0.01	**18.4**
#6	10.1 ± 1.5 ^{d}	0.22	6.78 ± 0.9 ^{d}	0.02	3.87 ± 0.5 ^{ef}	0.00	0.17 ± 0.0 ^{bc}	0.00	0.63 ± 0.2^{b}	0.00	1.00 ± 0.1 ^{e}	0.00	0.28 ± 0.0 ^{g}	0.00	0.03 ± 0.0^{c}	0.01	**22.8**
#7	16.8 ± 1.1^{b}	0.02	9.52 ± 0.9 ^{abc}	0.74	6.09 ± 0.6 ^{ab}	0.99	0.20 ± 0.0^{b}	0.00	0.41 ± 0.0^{c}	0.50	1.60 ± 0.1 ^{bc}	0.74	0.37 ± 0.0 ^{bcd}	0.18	0.04 ± 0.0 ^{ab}	0.65	**35.0**
#8	21.3 ± 2.5 ^{a}	0.00	10.3 ± 1.0 ^{a}	0.10	5.73 ± 0.6 ab	1.00	0.06 ± 0.0 ^{def}	0.31	0.36 ± 0.0^{c}	0.90	1.67 ± 0.1 ^{abc}	0.99	0.38 ± 0.0 ^{abc}	0.42	0.04 ± 0.0 ^{ab}	0.60	**39.8**
#9	16.0 ± 0.2^{b}	0.08	10.4 ± 0.5 ^{a}	0.05	4.40 ± 0.3 ^{de}	0.01	0.03 ± 0.0 ^{def}	0.85	0.33 ± 0.0^{c}	0.99	1.28 ± 0.0 ^{d}	0.00	0.33 ± 0.0^{f}	0.00	0.04 ± 0.0 ^{bc}	0.09	**32.9**
EtOH75	12.7 ± 1.2^{c}	1.00	8.70 ± 0.6 ^{bc}	1.00	5.86 ± 0.4 ^{ab}	1.00	ND	1.00	0.27 ± 0.0 ^{cd}	1.00	1.73 ± 0.1 ^{ab}	1.00	0.41 ± 0.0 ^{a}	1.00	0.05 ± 0.0 ^{a}	1.00	**29.7**
pH R^2	0.14		0.44		0.14		0.27		0.10		0.09		0.11		0.03		**0.03**

*yield was expressed as mean ± SD in dry skin from triplicate extraction; ^ p-Values of Dunnett's test compared with 75% ethanol (EtOH75); **Sum of phenolic acids (ellagic acid, gallic acid, and ferulic acid), flavan-3-ols (catechin and epicatechin), and flavonols (myricetin, quercetin, and kaempferol); ND, not detected; Extraction yield in the same column not connected by same letter are significantly different (ANOVA with Student's t test). pH R^2 indicates the correlations between pH of NDES and extraction yield of phenolic acids, flavonols, and flavan-3-ols extracted from the grape skin.

cranberry pomace [14]. This suggested that NDES #1 may selectively extract ellagic acid or ellagitannins from food matrix that also contain anthocyanidins. Such selectivity may be attributed to differences in the molecular interactions between the NDES and specific phenolic classes. Figure S1 (panel A) shows the HPLC chromatogram of gallic acid, ellagic acid and ferulic acid extracted from grape skin by NDES #1 and detected at 260 nm. The 75% ethanol extracted 12.7 ± 1.2 mg of ellagic acid per gram of grape skin. The lowest extraction yield of ellagic acid was observed in NDES #4 at 7.44 ± 0.6 mg/g. The extraction yield of gallic acid by NDES #9, #8, #1, #4, #7 and #3 were comparable and significantly higher than 75% ethanol. The highest amount of gallic acid was extracted by NDES #9 at 10.4 ± 0.5 mg/g, whereas the lowest amount of 5.55 ± 0.1 mg/g was extracted by NDES #5. The highest amount of ferulic acid was extracted by NDES #1 at 6.32 ± 0.7 mg/g and the lowest amount was extracted by NDES #5 at 3.11 ± 0.0 mg/g. Furthermore, there was no significant difference between NDES #1 and the 75% ethanol in extracting ferulic acid (Table 2).

The highest amount of catechin and epicatechin were extracted by NDES #3 at 0.61 ± 0.1 mg/g and 0.89 ± 0.1 mg/g, respectively (Table 2). Meanwhile, NDES #3 and #6 extracted significantly greater amounts of epicatechin than 75% ethanol. Figure S2 (panel A) shows the HPLC chromatogram of catechin and epicatechin extracted by NDES #3 from the grape skin. However, catechin was not detected in the 75% ethanol extract. The lowest amounts of catechin (0.02 mg/g) and epicatechin (0.14 mg/g) were extracted by NDES #2 and NDES #5, respectively.

Myricetin was the most abundant flavonols and kaempferol was the least. Dunnett's test revealed that NDES and 75% ethanol were comparable in extracting myricetin, quercetin and kaempferol (Table 2). The highest myricetin amount was extracted by NDES #1 (1.84 mg/g), followed by 75% ethanol (1.73 mg/g), and then NDES #8 (1.67 mg/g). The highest quercetin amount was extracted by 75% ethanol (0.41 mg/g), NDES #1 (0.40 mg/g), and NDES #8 (0.38 mg/g). In contrast, the lowest amounts of myricetin and quercetin were extracted by NDES #5 at 0.87 mg/g and 0.27 mg/g, respectively. This finding further emphasizes an overall weak ability of NDES #5 to extract polyphenols from the grape skin. The highest kaempferol amount was extracted by 75% ethanol (0.05 mg/g), and the lowest was extracted by NDES #5 and NDES#6 (0.03 mg/g). Figure S1 (panel B) shows the HPLC chromatogram of myricetin, quercetin and kaempferol extracted from grape skin by NDES #1 detected at 360 nm.

The highest sum amount of phenolic acids, flavonols, and flavan-3-ols was 40.7 mg/g extracted with NDES #1 followed by 39.8 mg/g extracted with NDES #8, whereas the lowest sum was 18.4 mg/g extracted by NDES #5 (Table 2).

The pH of NDES ranged between 0.3 and 3.3 (Table 1). The R-squared correlation between the pH of NDES and phenolic acids, flavonols, and flavan-3-ols yields were listed in Table 2. The lack of correlation between pH and extraction yields suggested pH did not impact extraction efficiency.

3.2. Polyphenols extracted by NDES from muscadine grape seeds

The overall extraction yields of phenolic acids, flavonols, and flavan-3-ols from grape seeds were noticeably lower than those from skins (Table 3). The most abundant extractable polyphenols in the seeds were catechin and epicatechin, whereas kaempferol was not detected. The complex seed matrix containing oil (13 %, w/w dry base) is a possible explanation of the low extractability of phenolic compounds from the grape seeds [19].

The highest amount of catechin was extracted by NDES #3 at 2.77 mg/g (Table 3). This yield was significantly higher than all other NDES and 75% ethanol. Figure S2 (panel B) shows the HPLC chromatogram of catechin and epicatechin extracted by NDES #3 from grape seeds. The lowest amount of catechin was extracted by NDES #5 at 0.30 mg/g. All NDES but NDES #1, #2 and #9 extracted significantly higher amounts of epicatechin than 75% ethanol (Table 3). The highest epicatechin concentrations were extracted by NDES #4 (0.71 mg/g) and NDES #5 (0.68 mg/g), whereas the lowest was extracted by 75% ethanol (0.11 mg/g).

Gallic acid was the most abundant extractable phenolic acid in the grape seeds, followed by ferulic acid and ellagic acid, respectively. The highest amount of gallic acid was extracted by NDES #4 at 0.45 mg/g, followed by NDES #9 and NDES #8. These NDES extracted significantly higher amounts of gallic acid than 75% ethanol. The lowest amount of gallic acid (0.2 mg/g) was extracted by NDES #3. The highest extraction

Table 3
Extraction yield of phenolic acids, flavonols, and flavan-3-ols from grape seeds by NDES and 75% ethanol.

NDES	Ellagic acid mg/g*	p^	Gallic acid mg/g	p	Ferulic acid mg/g	p	Catechin mg/g	p	Epicatechin mg/g	p	Myricetin mg/g	p	Quercetin mg/g	p	Sum** mg/g
#1	0.14 ± 0^{c}	0.63	0.34 ± 0.01^{c}	1.00	0.07 ± 0 ^{e}	0.00	0.43 ± 0.03 ^{de}	0.83	0.14 ± 0.02^{f}	0.20	0.13 ± 0 ^{d}	0.00	0.11 ± 0 ^{d}	0.00	**1.36**
#2	0.12 ± 0.01 ^{d}	0.94	0.31 ± 0.02^{c}	0.41	0.06 ± 0 ^{ef}	0.00	0.40 ± 0.02 ^{e}	0.13	0.13 ± 0.01 ^{fg}	0.70	0.12 ± 0.01 ^{e}	0.00	0.11 ± 0 ^{e}	0.00	**1.25**
#3	0.05 ± 0 ^{g}	0.00	0.20 ± 0 ^{e}	0.00	0.18 ± 0^{c}	0.00	2.77 ± 0.04 ^{a}	0.00	0.37 ± 0^{c}	0.00	0.17 ± 0.01 ^{bc}	0.14	0.13 ± 0.01^{b}	1.00	**3.87**
#4	0.13 ± 0.01 ^{cd}	1.00	0.45 ± 0 ^{a}	0.00	0.06 ± 0 ^{ef}	0.00	0.44 ± 0.02 ^{cde}	0.99	0.71 ± 0.01 ^{a}	0.00	0.12 ± 0 ^{e}	0.00	0.11 ± 0 ^{d}	1.00	**2.02**
#5	0.10 ± 0.01 ^{e}	0.00	0.23 ± 0.01 ^{d}	0.00	0.05 ± 0^{f}	0.00	0.30 ± 0.02^{f}	0.00	0.68 ± 0.02 ^{a}	0.00	0.08 ± 0.01^{f}	0.00	0.10 ± 0^{f}	0.01	**1.54**
#6	0.17 ± 0.01^{b}	0.00	0.32 ± 0.04^{c}	0.89	0.29 ± 0.03 ^{a}	0.00	0.45 ± 0.02 ^{bcd}	1.00	0.27 ± 0.01 ^{d}	0.00	0.16 ± 0.01^{c}	0.00	0.14 ± 0 ^{a}	0.00	**1.80**
#7	0.07 ± 0^{f}	0.00	0.23 ± 0.01 ^{d}	0.00	0.21 ± 0.01^{b}	0.00	0.49 ± 0.03^{b}	0.47	0.24 ± 0.01 ^{e}	0.00	0.18 ± 0.01 ^{ab}	0.94	0.12 ± 0^{c}	0.00	**1.54**
#8	0.13 ± 0 ^{cd}	0.98	0.41 ± 0.01^{b}	0.00	0.06 ± 0 ^{ef}	0.00	0.48 ± 0.01 ^{bc}	0.75	0.54 ± 0.02^{b}	0.00	0.11 ± 0.01 ^{e}	0.00	0.11 ± 0 ^{d}	0.00	**1.84**
#9	0.26 ± 0.01 ^{a}	0.00	0.44 ± 0.01 ^{ab}	0.00	ND	0.00	0.42 ± 0.04 ^{de}	0.54	0.14 ± 0.02^{f}	0.10	0.06 ± 0.01 ^{g}	0.00	0.11 ± 0^{f}	0.00	**1.43**
EtOH75	0.13 ± 0 ^{cd}	1.00	0.34 ± 0.0^{c}	1.00	0.11 ± 0 ^{d}	1.00	0.45 ± 0.04 ^{bcd}	1.00	0.11 ± 0.01 ^{g}	1.00	0.18 ± 0.01 ^{a}	1.00	0.12 ± 0^{c}	0.00	**1.44**
pH R^2	0.40		0.62		0.37		0.11		0.05		0.29		0.13		**0.12**

* yield was expressed as mean ± SD in dry seeds from triplicate extraction; ^ *p*-Values of Dunnett's test compared with 75% ethanol (EtOH75); **Sum of ellagic acid, gallic acid, ferulic acid, catechin, epicatechin, myricetin and quercetin; ND, not detected; Extraction yield in the same column not connected by the same letter are significantly different (ANOVA Student's *t* test). Kaempferol was not detected in all NDES and 75% ethanol. pH R^2 indicates the correlations between pH of NDES and extraction yield of phenolic acids, flavonols, and flavan-3-ols extracted from the grape seed.

yield of ellagic acid was obtained by NDES #9 (0.26 mg/g) followed by NDES #6 (0.17 mg/g), which were significantly higher than 75% ethanol. Similarly, NDES #3 extracted the lowest ellagic acid amount at 0.05 mg/g. In addition, NDES #6, #7 and #3 extracted significantly higher amounts of ferulic acid than 75% ethanol. The lowest ferulic acid extraction yield was 0.5 mg/g by NDES #5. Furthermore, ferulic acid was not detected in NDES #9 extract. This was likely because that the solubility of ferulic acid was lower in NDES #9 than in other NDES.

The highest myricetin extract was obtained by 75% ethanol and NDES #7 at 0.18 mg/g, which were higher than all NDES. The highest quercetin extraction yield was by NDES #6 (0.14 mg/g) and NDES #3 (0.13 mg/g) and both NDES were better than 75% ethanol. Similarly, the pH of NDES did not influence the extraction yield as indicated by the low correlation (R-squared) between the pH of NDES and yields of phenolic acids, flavonols, and flavan-3-ols listed in Table 3.

3.3. Principal component analysis

Principal component analysis (PCA) was performed to associate the extraction yield of various phenolic compounds in grape skin and seeds with NDES and 75% ethanol (Fig. 1). The PCA was performed on a correlation matrix to detect a possible selectivity of some NDES towards extracting specific phenolic compounds or groups. About 85% of the variance of skin data was explained by principal components 1 and 2. The loading plot (Fig. 1B) shows a high correlation between phenolic acids (ellagic acid, gallic acid, ferulic acid) and flavonols (myricetin, quercetin, and kaempferol). To extract these groups, the best solvents are NDES #1, #8, #7, and 75% ethanol as shown in the score plot (Fig. 1A). Meanwhile, catechin and epicatechin appeared segregated from the rest of the phenolic groups. As shown in Fig. 1A, NDES #3 was selective to extract catechin and epicatechin from grape skins. This was an interesting observation because NDES#3 was among the least effective NDES to extract proanthocyanidins, which are oligomers and polymers of catechin and epicatechin [14]. This suggested that NDES #3 may be selective to proanthocyanidins of smaller molecular size. The clustering of phenolic compounds on the loading plot of the skin (Fig. 1B) was different from the seed (Fig. 1D) regardless of the low yields of these compounds in the grape seeds. The first and second principal components explained about 73% of the variance of seed data. Quercetin, myricetin, and ferulic acid were extracted more efficiently by NDES #6, #7, and 75% ethanol, as shown in the score plot (Fig. 1C). Ellagic acid and gallic acid were extracted more effectively by NDES #9. Once again, catechin was extracted with the highest efficiency by NDES #3 which was similar to that observed with grape skins. Epicatechin was extracted with higher efficiency by NDES #5, #4, and NDES #8.

3.4. Extraction optimization of phenolic acids and flavonols from muscadine grape skins and ANN prediction modeling

Choline chloride: levulinic acid: ethylene glycol 1:1:2 (NDES #1) showed the highest extraction yield for ellagic acid, and therefore was chosen for further optimization and prediction. Impacts of four factors, including water content, ultrasonication time, solid-to-solvent ratio, and extraction temperature were assessed for the extraction of phenolic acids and flavonols. Furthermore, four levels for each extraction factor were applied in a total of 40 randomized runs. The experimental extraction yield of ellagic acid, gallic acid, ferulic acid, myricetin, and quercetin, along with the sum of these five, are shown in Table 4. Overall, the range of extraction yield difference between the lowest and the highest was relatively large for phenolic acids. For example, the lowest yield for ellagic acid was 9.03 mg/g (run #17) and the highest was 25.3 mg/g (run #15), which resulted in a difference of 16.2 mg/g

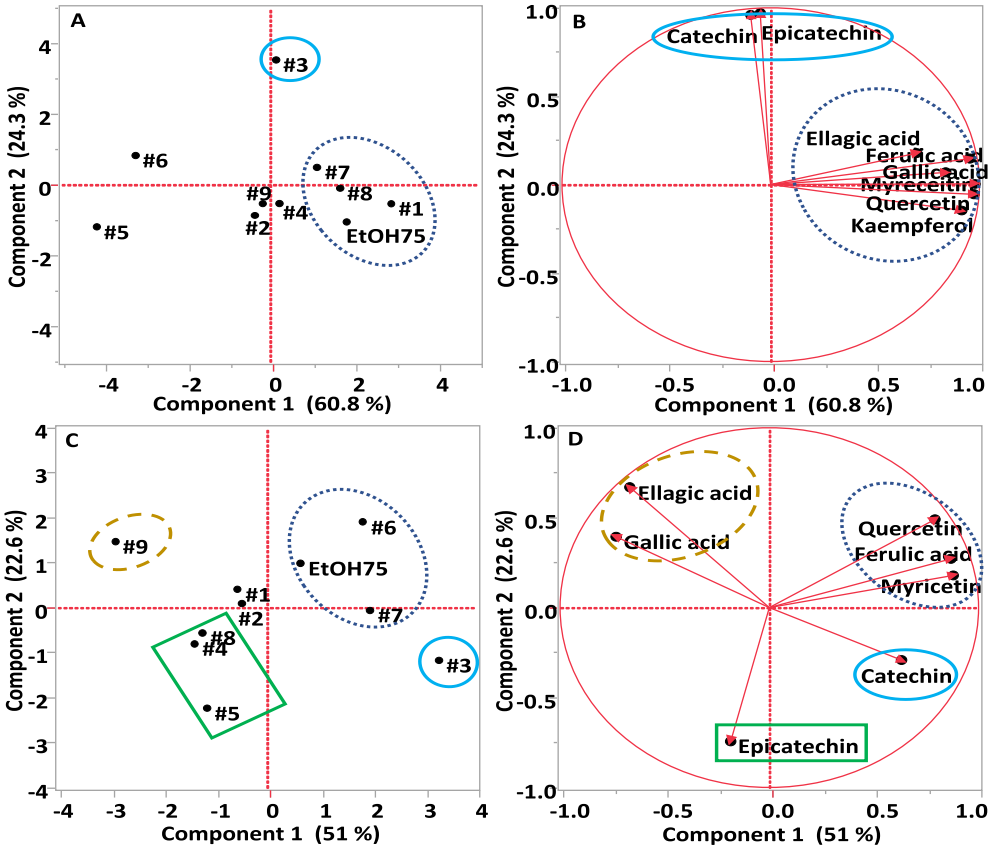

Fig. 1. Principal component analysis score plot (A) and loading plot (B) for NDES (#1-#9) and 75% ethanol (EtOH75) extraction of phenolic compounds from grape skins, score plot (C) and loading plot (D) for NDES (#1-#9) and 75% ethanol (EtOH75) extraction of phenolic compounds from grape seeds. Color-coded clusters on panel (B) and (D) shows the phenolic compounds with their respective best extraction solvents (NDES/75% ethanol) on panel (A) and (C).

Table 4
Experimental extraction yield of polyphenols from grape skin under different conditions using NDES #1 and predicted yield of ellagic acid by ANN.

Run Order	Water content	Ultrasoundtime	Solid-to-solvent ratio	Temperature	Ellagic acid		Gallic acid	Ferulic acid	Myricetin	Quercetin	SUM**
					Exp*	Prd^	Exp	Exp	Exp	Exp	Exp
	(mL/100 mL)	(min)	1:X (g:mL)	(°C)	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g
1	45	35	20	40	20.0	20.0	15.3	6.33	8.47	1.58	51.7
2	60	35	20	60	24.5	24.5	18.6	9.21	**10.1**	**1.87**	64.3
3	35	25	5	30	15.8	15.7	14.5	15.0	7.71	1.21	54.2
4	35	5	20	30	22.3	22.4	15.6	6.84	8.90	1.61	55.3
5	35	25	20	60	23.8	23.8	18.3	8.32	9.45	1.74	61.6
6	15	35	20	60	23.5	23.5	16.9	7.53	9.06	1.64	58.6
7	35	15	15	40	18.3	18.3	14.5	7.62	7.59	1.34	49.4
8	35	15	20	50	18.6	18.7	14.4	3.74	7.43	1.42	45.6
9	35	35	5	50	15.4	15.4	15.7	16.4	8.27	1.27	57.0
10	60	5	10	40	18.8	18.8	15.6	14.1	8.57	1.39	58.5
11	60	25	20	50	17.1	17.1	15.2	4.37	7.68	1.43	45.8
12	60	35	15	30	18.2	18.3	14.8	8.95	8.28	1.46	51.7
13	45	5	15	40	18.3	18.3	13.8	8.03	7.77	1.38	49.3
14	15	25	20	40	12.7	12.6	8.81	*ND*	4.67	*0.38*	26.6
15	45	25	10	60	**25.3**	**25.3**	18.3	16.6	9.70	1.56	**71.5**
16	45	25	10	50	18.6	18.7	14.4	11.2	7.61	1.27	53.1
17	15	25	5	30	*9.03*	9.07	7.07	*ND*	3.91	0.66	*20.7*
18	60	25	15	30	19.8	19.7	16.2	10.3	8.91	1.55	56.8
19	45	15	20	30	20.0	20.0	14.8	6.43	8.53	1.60	51.4
20	35	5	10	30	19.9	20.0	15.1	13.0	8.34	1.37	57.7
21	60	5	20	50	19.6	19.7	15.3	5.36	8.27	1.56	50.1
22	60	5	5	60	20.1	20.1	18.3	**19.2**	9.16	1.41	68.2
23	60	35	5	50	15.2	15.2	14.0	13.5	7.08	1.09	50.9
24	60	25	15	60	9.82	9.84	**18.6**	10.7	9.03	1.57	49.7
25	60	15	5	40	16.2	16.2	14.6	15.1	7.72	1.18	54.8
26	35	35	10	40	17.8	17.8	14.7	11.3	7.66	1.26	52.7
27	60	15	10	30	18.9	18.8	16.1	14.2	9.03	1.46	59.7
28	45	35	5	30	11.6	11.8	11.3	11.8	6.40	1.00	42.1
29	15	5	20	30	12.8	12.8	9.10	*ND*	5.39	1.11	28.4
30	35	5	5	60	19.8	19.8	17.0	19.1	9.34	1.45	66.7
31	15	5	5	50	14.5	14.5	11.1	11.0	5.98	0.95	43.5
32	35	35	15	60	24.1	24.1	18.4	11.8	9.31	1.62	65.2
33	45	5	15	50	16.6	16.5	14.5	6.96	7.42	1.33	46.8
34	15	15	15	50	12.4	12.4	8.60	*ND*	4.62	0.94	26.6
35	15	5	15	60	18.9	18.8	13.3	6.76	7.24	1.33	47.5
36	15	15	10	60	18.2	18.3	15.2	12.4	8.16	1.38	55.3
37	15	35	15	30	13.5	13.4	10.3	3.07	5.73	1.10	33.7
38	15	35	10	50	12.2	12.2	8.62	4.24	4.60	0.84	30.5
39	45	15	5	60	22.0	22.0	16.5	17.6	8.64	1.33	66.2
40	15	25	5	40	9.80	9.72	*6.63*	5.64	*3.79*	0.64	26.5

Data are shown as mg phenolic acids or flavonols per gram of dry grape skin; * Experimental yield; ^ Predicted yield by ANN; **Sum of experimental ellagic acid, gallic acid, ferulic acid, myricetin and quercetin; ND, not detected. The highest yield and lowest yield for each compound were bolded and italicized, respectively.

(run #17). Moreover, the lowest sum of yield was 20.7 mg/g, and the highest was 71.5 mg/g. This illustrates the significant impacts of the different levels of each extraction factor on extraction yield.

Run #15 extracted the highest amount of ellagic acid. The extraction condition of run #15 were 45 mL /100 mL water content, 25 min of ultrasonication, 1:10 (g:mL) solid-to-solvent ratio and extraction temperature of 60 °C. Figure S3 shows the HPLC chromatogram of optimized phenolic acids extracted from grape skin by NDES #1 (run#15 in Table 4). The highest gallic acid (18.7 mg/g) was achieved using the extraction conditions in run #24 and the lowest was 6.63 mg/g using run #40. For ferulic acid, run #22 extracted the highest amount at 19.2 mg/g, whereas no ferulic acid was detected in runs #14, #17, #29, and #34. Run #22 was extracted with 60 mL /100 mL water content, 5 min of ultrasonication, 1:5 for solid-to-solvent ratio and extraction temperature of 60 °C. Run #2 extracted the highest myricetin (10.1 mg/g) and quercetin (1.87 mg/g). The extraction conditions of run #2 were 60 mL /100 mL water content, 35 min of ultrasonication, 1:20 for solid-to-solvent ratio and extraction temperature of 60 °C. The lowest myricetin yield (3.79 mg/g) was extracted by run#40.

The contour plots in Fig. 2 demonstrate the effect of extraction parameters (X_1, X_2, X_3, and X_4) on the predicted yield of ellagic acid extracted by NDES #1 from the grape skin. The predicted yields of ellagic acid in Table 4 were utilized to construct these count plots. Each panel illustrates the impact of 2 extraction parameters. The contour lines are labeled with the yield of ellagic acid (mg/g). The optimum predicted water content was about 35–45 mL/100 mL NDES, as shown in Fig. 2B and 2C. Longer ultrasonication time increased the yield of ellagic acid (Fig. 2D and 2E), indicating a critical role of sonication in NDES extraction. During the extraction, mixing grape skins or seeds with NDES introduced particles and gas, which added acoustic cavitation sites for ultrasounds to generate numerous small bubbles in the NDES. The imploding of these bubbles led to extreme temperature, pressure differential, high shear force, macro-turbulences, and micro-mixing, which effectively agitated NDES to accelerate mass diffusion and transfer. When cavitation bubbles imploded on the surface of grape seed or skin particles, the resultant micro-jets and inter-particle collisions led to surface peeling, erosion, particle breakdown, sonoporation, and cell disruption [20]. All these mechanical effects of ultrasound-induced cavitation intensified the penetration of NDES to the cell interior so that intercellular phenolics from food matrix were transferred into solvents.

The optimum solid-to-solvent ratio was 1:10, as indicated by Fig. 2B, 2D, and 2F. Lastly, higher extraction temperatures up to 60 °C seem to have a positive effect on the ellagic acid extractability as shown in Fig. 2C, 2E, and 2F. This suggests a direct relationship between extraction temperature and the yield of ellagic acid extracted from the grape skin.

Ellagic acid extraction yields (Table 4) were analyzed for prediction

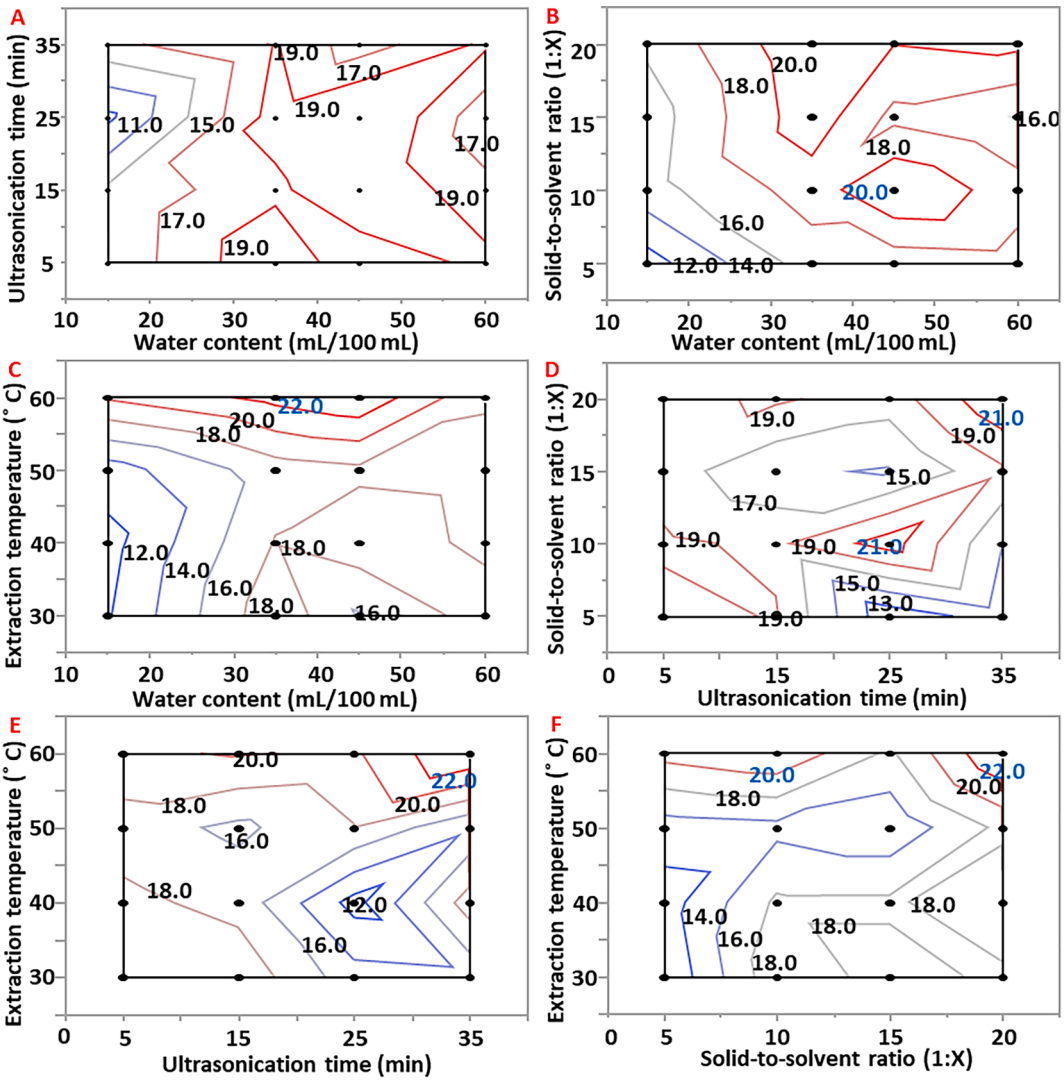

Fig. 2. Contour plots showing the effects of (A) water content (X_1) and ultrasonication time (X_2), (B) water content (X_1) and solid-to-solvent ratio (X_3), (C) water content (X_1) and extraction temperature (X_4), (D) ultrasonication time (X_2) and solid-to-solvent ratio (X_3), (E) ultrasonication time (X_2) and extraction temperature (X_4), (F) solid-to-solvent ratio (X_3) and extraction temperature (X_4) on ellagic acid predicted yield from grape skin using NDES #1.

modeling using artificial neural networking. The experimental data were randomly split into a training set and a validation set. The reason to include a validation set by the statistical software is to suppress over-fitting. To predict ellagic acid yield (Y), the same four independent extraction factors (X_1, X_2, X_3 and X_4), 1–2 hidden layers with a different number of neurons and three activation functions were assessed. The applied activation functions were hyperbolic tangent, linear, and gaussian. Next, the datasets were trained until a high R-squared value for both training and validation was reached. The prediction data and a model were generated. The best ANN structure was chosen by analyzing the four inputs (X_1, X_2, X_3 and X_4) with one hidden layer using the gaussian function with ten neurons (Figure S5). The R-squared of training and validation sets were 0.99, whereas the RASE and AAE of the model were 0.062 and 0.044, respectively. The R-squared of the ANN validation of ellagic acid in this study (0.99) was higher than the ANN validation of procyanidins (0.95) and anthocyanins (0.91) in a previous study [14]. However, this increase in R^2 may be attributed to better generated model fit of the data in this study, which could be due to the smaller experimental errors. The predictive ANN models for the extraction of ellagic acid using NDES #1 were shown as equation 3–13:

$$(H1\_1) = Exp(-(0.5*((-3.95) + 0.01*X1 + 0.09*X2 + 0.42*X3 + -0.15*X4)^2)) \quad (3)$$

$$(H1\_2) = Exp(-(0.5*((-8.88) + -0.05*X1 + 0.03*X2 + 0.14*X3 + 0.16*X4)^2)) \quad (4)$$

$$(H1\_3) = Exp(-(0.5*((-7.55) + 0.02*X1 + -0.08*X2 + 0.41*X3 + 0.09*X4)^2)) \quad (5)$$

$$(H1\_4) = Exp(-(0.5*((-0.53) + 0.11*X1 + -0.06*X2 + -0.09*X3 + -0.02*X4)^2)) \quad (6)$$

$$(H1\_5) = Exp(-(0.5*((-6.65) + -0.12*X1 + 0.19*X2 + 0.15*X3 + 0.11*X4)^2)) \quad (7)$$

$$(H1\_6) = Exp(-(0.5*((-0.23) + -0.02*X1 + 0.12*X2 + 0.24*X3 + -0.04*X4)^2)) \quad (8)$$

$$(H1\_7) = Exp(-(0.5*(16.85 + -0.08*X1 + -0.052*X2 + -0.10*X3 + -0.17*X4)^2)) \quad (9)$$

$$(H1\_8) = Exp(-(0.5*((-4.00) + -0.06*X1 + -0.05*X2 + 0.14*X3 + 0.12*X4)^2)) \quad (10)$$

$$(H1.9) = Exp(-(0.5*((-2.66) + 0.01*X1 + 0.00*X2 + 0.08*X3 + 0.04*X4)^2)) \quad (11)$$

$$(H1\_10) = Exp(-(0.5*(20.28 + -0.15*X1 + 0.11*X2 + -0.38*X3 + -0.21*X4)^2)) \quad (12)$$

$$PredictedY = 16.28 + 4.518*H1\_1 + 2.614*H1\_10 + -3.897*H1\_2 + 5.149*H1\_3 + 6.748*H1\_4 + -4.715*H1\_5 + 2.394*H1\_6 + 4.458*H1\_7 + -0.569*H1\_8 + -5.693*H1\_9 \quad (13)$$

The model predicted yields of ellagic acid are listed in Table 4. Using extraction conditions of run #15 would predict the yield of ellagic acid to be 25.3 mg/g, which matched the observed value of 25.3 mg/g. This generated model (equation #12) perhaps is unique to using only NDES #1 with cited extraction conditions and grape skin. However, this illustrates the high ability of ANN for predicting extraction yield. One of

Table 5
Experimental extraction yield of catechin and epicatechin from grape seeds using NDES #3 under different conditions and predicted values of catechin + epicatechin by ANN.

Run Order	Water content	Ultrasoundtime	Solid-to-solvent ratio	Temperature	Catechin	Epicatechin	Catechin + Epicatechin	
					Exp*	Exp	Exp	Prd^
	(mL/100 mL)	(min)	1:X (g:mL)	(°C)	mg/g	mg/g	mg/g	mg/g
1	45	35	20	40	6.95	1.78	8.73	8.70
2	60	35	20	60	5.85	1.13	6.98	7.10
3	35	25	5	30	2.37	0.66	3.03	2.81
4	35	5	20	30	2.20	0.69	2.89	3.06
5	35	25	20	60	2.51	0.67	3.18	3.30
6	15	35	20	60	1.49	0.41	1.90	2.02
7	35	15	15	40	3.50	0.81	4.31	4.98
8	35	15	20	50	2.14	0.57	2.71	2.59
9	35	35	5	50	1.43	0.38	1.81	1.77
10	60	5	10	40	4.55	1.26	5.81	5.96
11	60	25	20	50	6.07	1.46	7.53	7.45
12	60	35	15	30	5.18	1.17	6.35	6.31
13	45	5	15	40	5.45	**3.27**	8.72	8.25
14	15	25	20	40	1.59	0.42	2.01	1.94
15	45	25	10	60	4.29	0.78	5.07	4.85
16	45	25	10	50	4.29	0.85	5.14	5.15
17	15	25	5	30	0.64	0.15	0.79	0.69
18	60	25	15	30	5.71	1.48	7.19	7.54
19	45	15	20	30	4.83	1.37	6.20	6.06
20	35	5	10	30	2.22	0.57	2.79	2.74
21	60	5	20	50	4.21	1.01	5.22	5.48
22	60	5	5	60	3.58	0.77	4.35	4.09
23	60	35	5	50	4.25	0.90	5.15	5.24
24	60	25	15	60	5.62	0.99	6.61	6.18
25	60	15	5	40	4.59	1.28	5.87	5.84
26	35	35	10	40	2.68	0.66	3.34	3.42
27	60	15	10	30	3.90	0.96	4.86	4.79
28	45	35	5	30	*0.22*	*0.07*	*0.29*	*0.36*
29	15	5	20	30	**7.98**	1.86	**9.84**	**9.60**
30	35	5	5	60	0.73	0.18	0.91	1.03
31	15	5	5	50	1.24	0.25	1.49	1.37
32	35	35	15	60	3.35	0.63	3.98	4.01
33	45	5	15	50	3.70	0.84	4.54	4.44
34	15	15	15	50	1.45	0.47	1.92	2.12
35	15	5	15	60	1.17	0.39	1.56	1.50
36	15	15	10	60	1.65	0.37	2.02	1.77
37	15	35	15	30	0.59	0.19	0.78	0.86
38	15	35	10	50	0.59	0.16	0.75	0.84
39	45	15	5	60	2.88	0.57	3.45	3.69
40	15	25	5	40	1.80	0.49	2.29	2.49

Data are shown as mg catechin and epicatechin /g dry grape seed; * Experimental yield; ^ Predicted yield by ANN. The highest yield and lowest yield for each compound were bolded and italicized, respectively. The highest yield and lowest yield for each compound were bolded and italicized, respectively.

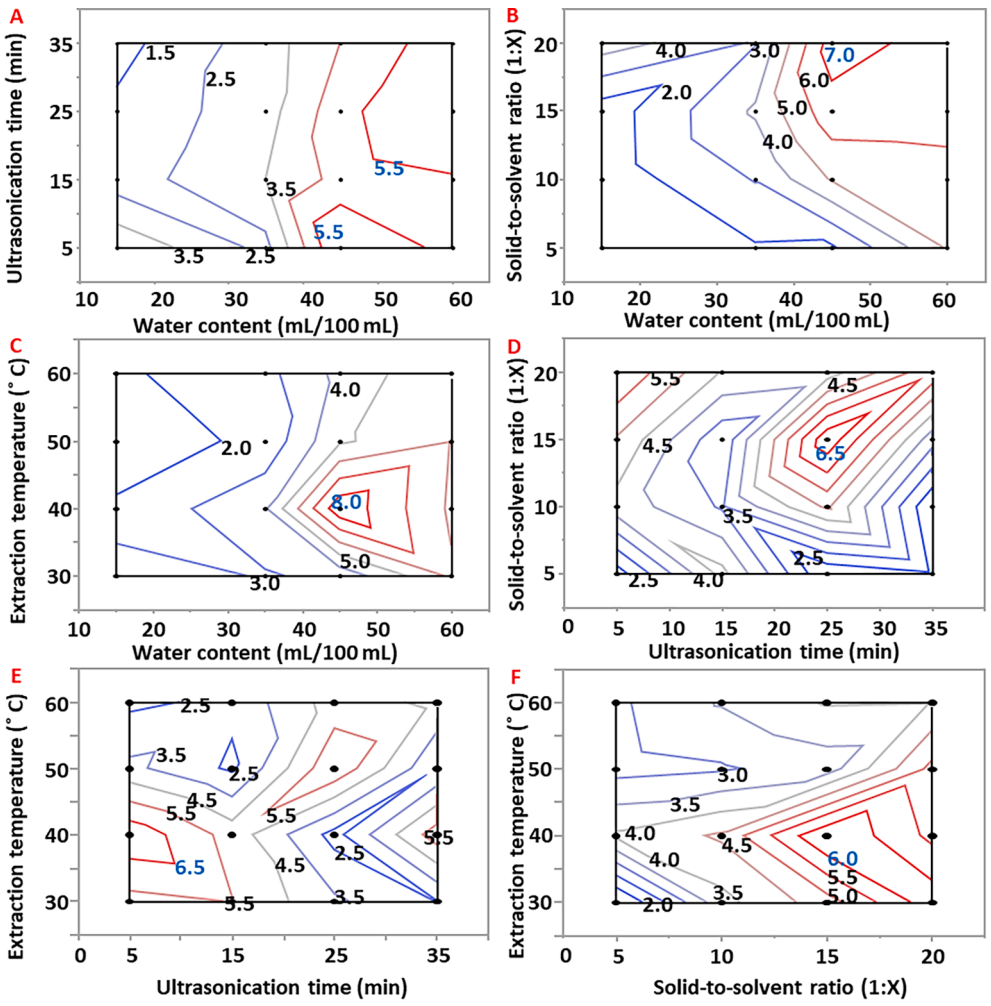

Fig. 3. Contour plots showing effects of (A) water content (X_1) and ultrasonication time (X_2), (B) water content (X_1) and solid-to-solvent ratio (X_3), (C) water content (X_1) and extraction temperature (X_4), (D) ultrasonication time (X_2) and solid-to-solvent ratio (X_3), (E) ultrasonication time (X_2) and extraction temperature (X_4), (F) solid-to-solvent ratio (X_3) and extraction temperature (X_4) on catechin + epicatechin predicted yield from grape seeds by NDES #3.

the drawbacks of ANN compared to conventional optimization and prediction methods is that it does not truly reveal much about which independent parameter (input) significantly contributed to the overall extraction performance (output). Also, ANN does not rank the inputs based on their greater capacities to predict the output.

3.5. Extraction optimization of catechin and epicatechin from muscadine grape seeds and ANN prediction modeling

Choline chloride: proline: malic acid 1:1:1 (NDES #3) was optimized for the extraction of catechin and epicatechin from grape seeds. NDES #3 was selected for optimization and prediction because of its high capability of extracting catechin and epicatechin from both grape skin and seeds. The yields of catechin and epicatechin and the sum of the two are shown in Table 5. The highest sum of catechin and epicatechin was 9.84 mg/g by run#29 and the lowest was 0.29 mg/g by run #28.

The highest yield of catechin was 7.98 mg/g extracted by NDES #3 in run #29. The extraction conditions of run #29 consisted of 15 mL water/100 mL water content, 5 min of ultrasonication, 1:20 for solid-to-solvent ratio, and the extraction temperature of 30 °C. Figure S4 shows the HPLC chromatogram of catechin and epicatechin extracted from grape skin by NDES #3 (run#29 in Table 5). The lowest catechin yield was 0.22 mg/g extracted in run #28. The range between the highest and lowest yield of catechin was 7.76 mg/g.

The highest epicatechin yield was 3.27 mg/g extracted in run #13 using 45 mL water/100 mL water content, 5 min of ultrasonication, 1:15 for solid-to-solvent ratio and the extraction temperature of 40 °C. Likewise, the lowest epicatechin yield was achieved by run #28 at 0.07 mg/g. The range of epicatechin extraction yield was 3.2 mg/g.

The contour plots in Fig. 3 illustrate the effects of the extraction parameters (X_1, X_2, X_3, and X_4) on the predicted yield of catechin and epicatechin extracted by NDES #3 from grape seeds. The counters on panels A-F are labeled with the predicted yield of sum of catechin and epicatechin (mg/g). Overall, the extraction parameters affected the predicted yield of catechin and epicatechin differently. Fig. 3B shows the impact of water content (X_1) and solid-to-solvent ratio (X_3) on the yield of catechin + epicatechin extraction from grape seeds. For example, 45% of water in the NDES and 1:20 solid-to-solvent ratio would result in a 7 mg of catechin + epicatechin per gram of grape seed. Like the ellagic acid extraction, water content of 45–50 mL / 100 mL NDES would result in a significantly higher yield of catechin and epicatechin as demonstrated in Fig. 3A, 3B and 3C. Ultrasonication time of 10–15 min would increase the extractability of sum of catechin and epicatechin (Fig. 3A, 3D and 3E). A solid-to-solvent ratio of 1:15–1:20 would result in the

highest yield of sum of catechin and epicatechin as indicated in Fig. 3B, 3D and 3F. Lastly, the highest yield was predicted with applied extraction temperature of 30–40 °C, as shown in Fig. 3C, 3E and 3F. This suggests an inverse relationship between extraction temperature and yield of catechin and epicatechin conversely to that predicted with optimization of ellagic acid extraction. Furthermore, this relationship indicated that catechin and epicatechin might degrade with higher extraction temperature.

The experimental sum of catechin and epicatechin data in Table 5 were analyzed by ANN for prediction. Similar to the ellagic acid, the 40 data points were spilt into a training set and a validation set. The optimum ANN structure consisted of four inputs (X_1, X_2, X_3 and X_4) with one hidden layer using the gaussian function (10 neurons) to predict the sum of catechin and epicatechin (Figure S5). The R-squared of training and validation datasets were 0.99, whereas the RASE and AAE of the model were 0.20 and 0.15, respectively. The predictive ANN models for the extraction of sum of catechin and epicatechin using NDES #3 were shown as equation 14–24:

$$(H1\_1) = Exp(-(0.5*(8.548 + -0.070*X1 + -0.118*X2 + -0.2413*X3 + -0.007*X4)^2) \quad (14)$$

$$(H1\_2) = Exp(-(0.5*((-14.83) + 0.085*X1 + 0.054*X2 + 0.542*X3 + 0.066*X4)^2) \quad (15)$$

$$(H1\_3) = Exp(-(0.5*((-0.499) + 0.087*X1 + -0.074*X2 + -0.153*X3 + -0.029*X4)^2) \quad (16)$$

$$(H1\_4) = Exp(-(0.5*((-6.061) + 0.044*X1 + -0.141*X2 + 0.140*X3 + 0.109*X4)^2) \quad (17)$$

$$(H1\_5) = Exp(-(0.5*((-2.941) + 0.095*X1 + -0.008*X2 + -0.073*X3 + 0.037*X4)^2) \quad (18)$$

$$(H1\_6) = Exp(-(0.5*(1.000 + 0.004*X1 + -0.044*X2 + 0.084*X3 + -0.016*X4)^2) \quad (19)$$

$$(H1\_7) = Exp(-(0.5*(7.273 + 0.024*X1 + 0.016*X2 + -0.125*X3 + -0.171*X4)^2) \quad (20)$$

$$(H1\_8) = Exp(-(0.5*((-4.742) + 0.050*X1 + 0.039*X2 + 0.027*X3 + 0.000*X4)^2) \quad (21)$$

$$(H1\_9) = Exp(-(0.5*((-4.838) + 0.038*X1 + 0.144*X2 + -0.204*X3 + 0.034*X4)^2) \quad (22)$$

$$(H1\_10) = Exp(-(0.5*((-8.718) + 0.137*X1 + 0.034*X2 + -0.167*X3 + 0.122*X4)^2) \quad (23)$$

$$PredictedY = 6.139 + -2.613*H1\_1 + 2.767*H1\_10 + 1.564*H1\_2 + -0.922*H1\_3 + 1.796*H1\_4 + -3.811*H1\_5 + -5.126*H1\_6 + 1.947*H1\_7 + 2.690*H1\_8 + 2.245*H1\_9 \quad (24)$$

The ANN model predicted the yields of sum of catechin and epicatechin as presented in Table 5. The predicted yield of the sum of catechin and epicatechin with extraction conditions in run#29 using NDES #3 was 9.60 mg/g, which was comparable to the observed value of 9.84 mg/g.

4. Conclusion

Current findings presented further evidence on the effectiveness of NDES abilities to extract polyphenols from food industry by-products. The outcomes supported the hypothesis of a superior ultrasound-assisted extraction of NDES over 75% ethanol. NDES effectively extracted three phenolic acids, two flavonols, and three flavan-3-ols from grape skins and seeds. NDES #1 was the most effective NDES to extract ellagic acid, whereas NDES #3 was notably selective towards extracting catechin and epicatechin. A noticeable drawback of NDES is their high viscosity, which presents challenges during handling and recovery. In the present study, artificial neural networking, regardless of its outcome limitations, demonstrated a practical approach for predictive modeling. NDES are robust media to recover phytochemicals from food systems. Some NDES also present a less toxic solvent to study these phytochemicals in living cells [21,22]. At last, natural deep eutectic solvents are effective alternative extraction media to organic solvents.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Mohammed Alrugaibah: Data curation, Formal analysis, Writing - original draft. **Taylor L. Washington:** Writing - review & editing. **Yavuz Yagiz:** Investigation, Writing - review & editing. **Liwei Gu:** 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.

Acknowledgment

The first author is grateful for the Ph.D. scholarship provided by Qassim University, KSA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105773.

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