Original Article

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New solvents for extracting gallic acid from chestnut shells: deep eutectic solvents

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Abstract: Chestnut (*Castanea sativa* Mill.) shell, a byproduct of chestnut processing, is a valuable source of phenolic compounds that have a range of industrial applications. This study investigated the optimization of gallic acid extraction from the outer shell of the chestnut using ultrasound-assisted extraction (UAE) and deep eutectic solvents (DES). A Box–Behnken design (BBD) was employed to determine optimal extraction conditions, considering variables such as extraction time, temperature, water content, liquid-to-solid ratio, material treatment, and DES type. Results indicate that baked chestnut shell samples and choline chloride:ethylene glycol (1:2) DES yielded the highest gallic acid content, with optimal conditions of 70 °C, 35 min, 20% water content, and a 10:1 liquid-to-solid ratio. This study highlights the environmental and economic advantages of DES-based UAE, supporting its use as a sustainable method for extracting bioactive compounds for application in the food, pharmaceutical, and cosmetic industries.
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Key words: *Castanea sativa* Mill.; green extraction; ultrasound-assisted extraction; phenolic compounds

Introduction

The chestnut tree (*Castanea sativa* Mill.), a member of the Fagaceae (beech) family, is widely found in southern Europe and Asia. The chestnut fruit is of considerable economic value, mainly because of its nutritional qualities and health benefits. Chestnut production is concentrated in Mediterranean countries and the fruit is consumed either raw or cooked. It is mainly used as candied chestnut, chestnut purée, and, in recent years, gluten-free flour due to its low fat content. Chestnut processing generates byproducts amounting to approximately 10% (w/w) of each fruit, including the inner shell, outer shell, and spiny bur.[1,2] These byproducts can contribute to the creation of new value-added products and economic resources while meeting the sustainability requirements of the industry. They can serve as natural sources of bioactive compounds in the nutraceutical, pharmaceutical, and cosmetic industries.[3–5]

As chestnut production and processing grows worldwide, the use of its byproducts is becoming increasingly important. For example, chestnut shells are known to be rich in phenolic compounds and have antioxidant properties, making them

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particularly valuable in the cosmetics industry as antiaging agents. Both traditional and modern extraction techniques can be used to extract useful compounds such as polyphenols from chestnut byproducts, offering potential health benefits in a number of areas. These benefits include antioxidant and anti-inflammatory effects.[6]

Gallic acid, a phenolic compound found in various plant species, is valued for its many applications in the food, pharmaceutical, and cosmetic industries. Known for its antioxidant, anti-inflammatory, and antimicrobial properties, gallic acid is widely employed as a preservative due to its ability to prevent the rancidity of oils and fats.[7] Derivatives of gallic acid, such as propyl and octyl gallates, are commonly used in the food industry due to their antioxidant properties, which extend shelf life and increase product stability.[8] Gallic acid has also been demonstrated to possess beneficial effects on cardiovascular, neuropsychological, and gastrointestinal disorders through its interaction with various biological pathways, including those regulating inflammation and cell apoptosis.[9]

In the field of cosmetics, gallic acid and its derivatives have gained attention for their ability to inhibit melanin production and combat oxidative stress. This renders them suitable for incorporation into skin and hair care products. Gallic acid is a popular ingredient in anti-aging, antiwrinkle, and skin-whitening substances due to its potent free-radical scavenging abilities.[10] When used in conjunction with heat it also provides a more benign alternative to traditional chemical hair-straightening treatments. It is also a key ingredient in natural hair dyes. The versatility and efficacy of gallic acid in a multitude of industrial applications underscore its significance, positioning it as a compelling subject for further investigation and development across a diverse range of sectors.[11]

Ultrasound-assisted extraction (UAE) has demonstrated considerable potential in the extraction of bioactive compounds from plant matrices, offering a means of reducing reliance on high solvent volumes and extreme temperatures, which are typical of conventional extraction techniques. By leveraging the phenomenon of cavitation, UAE more effectively disrupts cellular walls, thereby facilitating the release of valuable phytochemicals, including polyphenols, flavonoids, and other antioxidants. This method not only accelerates the extraction process but also safeguards the structural integrity of thermolabile compounds that may degrade under heat. Ultrasound-assisted extraction is particularly advantageous for recalcitrant biomaterials, for which pretreatments such as milling or enzymatic hydrolysis can further optimize the yield of extracted compounds and the antioxidant potential of the resulting extracts. By providing precise control over the conditions of the extraction process, UAE continues to evolve as a highly adaptable and eco-friendly approach in the field of natural product extraction.[12]

The conventional approach to the extraction of polyphenols and other bioactive compounds from plant matrices relies on the use of organic solvents, such as ethanol, methanol, and acetone. However, these solvents present a number of significant drawbacks, including high volatility, toxicity, poor biodegradability, flammability, and high cost. In response to these shortcomings, deep eutectic solvents (DES) have emerged as environmentally friendly alternatives. The use of naturally occurring components such as choline chloride and organic acids renders DES biodegradable. These solvents are formed through hydrogen bonding between hydrogen bond acceptors (HBAs) and donors (HBDs), resulting in stable and environmentally friendly systems. This renders DES an attractive option for green extraction processes, particularly in the context of food and medicinal applications, where they can facilitate the efficient extraction of bioactive constituents from various plant-based matrices including fruits, herbs, and medicinal plants. Their physicochemical properties – such as melting point, density, viscosity, surface tension, conductivity, refractive index, and pH – are largely governed by the interactions between HBAs and HBDs. Their unique eutectic behavior results in lower melting points than their individual components. Typically, DES exhibit high viscosity and low conductivity at room temperature. Viscosity and conductivity can be adjusted by changing temperature, molar ratios, or component structures. Deep eutectic solvents are often hygroscopic, with water content affecting their properties significantly by disrupting hydrogen bonding and enhancing ionic mobility. Their biodegradability also depends on the neutrality and chemical nature of the HBD and HBA used. These combined features make DES especially suitable for sustainable applications, particularly when solvent performance, polarity, and environmental safety are essential.[13] Deep eutectic solvent-based ultrasound-assisted extraction (UAE) also enhances sustainability by minimizing the use of solvents, labor, and energy. The full potential of DES in various industrial and environmental applications remains to be elucidated; however, they represent a significant advance in the pursuit of sustainable extraction solutions.[14,15]

It is important to employ innovative and sustainable methodologies in order to investigate the potential of chestnut byproducts and optimize the extraction of bioactive compounds. This study is concerned with the utilization of advanced extraction techniques, including UAE and DES, for the efficient isolation of phenolic compounds, in particular gallic acid, while ensuring minimal environmental impact.

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Material and methods

Material

This study used the outer shell (brown pericarp) of the European chestnut (*C. sativa*). Samples were harvested from Amasra, Bartin, Turkey in September 2021 and stored in a freezer until they were analyzed. Chestnut shells were obtained after subjecting whole chestnut fruits to different thermal treatments. In all cases, the edible kernel and inner shell were removed prior to extraction. Three different forms of the outer shell (brown pericarp) were used in this study: (S1) the untreated control sample, in which edible kernel was removed and the shell was used; (S2) boiled chestnut shell, obtained by boiling the whole chestnuts in tap water at 100 °C for 25 min before removing the shells; and (S3) baked chestnut shell, collected after baking the whole chestnuts in an oven at 150–200 °C for 25 min. After separation, all outer shells were ground using a home-type coffee grinder and stored in glass bottles until extraction.

Chemicals

The chemicals and reagents were of analytical grade. Choline chloride (ChCl) (98%), formic acid (FA) (98%) and acetic acid (AA) (100%) were purchased from Merck, Darmstadt, Germany. Ethylene glycol (EG, 99%) and gallic acid monohydrate (GA) (99%, Sigma 27645) were obtained from Sigma-Aldrich, St. Louis, MO, USA. The solvents used were of high-performance liquid chromatography (HPLC) grade and the water was ultrapure.

Preparation of deep eutectic solvents

In all DES mixtures, ChCl was used as the HBA, whereas formic acid, acetic acid, and ethylene glycol served as HBDs.

The molar ratio of HBA to HBD was maintained at (1:2). These components were selected based on their biodegradability, low toxicity, and capacity to form stable eutectic systems with ChCl.

Each HBD possesses distinct physicochemical characteristics. Formic and acetic acids are short-chain organic acids offering high polarity and acidity, which can enhance solubilization of phenolics through protonation and hydrogen bonding. Ethylene glycol, on the other hand, is a polyol with lower acidity and higher viscosity, contributing to a different solvation environment that may better stabilize phenolic structures.

The DES mixtures were prepared by heating the components at 70 °C with continuous stirring for 30 min until clear, homogeneous liquids formed. The resulting DESs were cooled to room temperature and stored in amber glass bottles to prevent degradation.

Conventional extraction

Conventional extraction was performed using a Soxhlet apparatus with methanol:water (95:5 v/v) and acetone:water (90:10 v/v). Five grams of sample was extracted with 300 mL of solvent for 6 h. Solvent aliquots were stored frozen until HPLC analysis.

Ultrasound-assisted liquid–solid extraction

Ultrasound-assisted liquid–solid extraction (UA-LSE) is a widely used and inexpensive method for the preparation of phenolic compounds. One gram of chestnut shell was placed in a glass tube with DES solution (10:1, 20:1, 30:1 mL g^{-1} and treated in an ultrasound bath. After the extraction, the aliquot was filtered and processed in a centrifuge to ensure that no suspended particles remained. The prepared DES solution was passed through a nonsterile polytetrafluoroethylene (PTFE) 25 mm diameter, 0.45 µm pore syringe filter and kept in a refrigerator at 4 °C until HPLC analysis.

Experimental design

In this study, a Box–Behnken design (BBD) was used to perform the experimental measurements. The BBD, a statistical experimental design developed by George E. P. Box and Donald Behnken in 1960, is a widely used method in response surface methodology (RSM).[16] This design is particularly valuable for optimizing process parameters and their levels efficiently.[17] Its ability to capture second-order behavior by varying parameters at three levels makes it a versatile tool for modeling complex systems.[18] By varying factors and levels systematically, the BBD enables researchers to efficiently explore the design space and identify optimal conditions.[19–21] By utilizing this design, researchers can determine the optimal levels of significant factors efficiently while minimizing the number of experimental runs.[22]

Six parameters including extraction time, extraction temperature, amount of water, liquid-to-solid ratio, material, and DES type were defined at three levels each to optimize the amount of gallic acid in the European chestnut (Table 1).

Characterization of gallic acid by HPLC analysis

The amount of gallic acid in the DES and solvent samples was determined using HPLC (Shimadzu, LC20AD, Shimadzu Corp., Kyoto, Japan). A reverse-phase HPLC equipped with

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Table 1. Levels of the BBD.

Codes	Parameters	Levels		
		−1	0	1
A	Extraction time (min.)	10	35	60
B	Extraction temperature (°C)	30	50	70
C	Water (%)	10	20	30
D	Liquid/solid ratio (mL g^{-1})	10	20	30
		1	2	3
E	Material	Control sample	Baked	Boiled
F	DES	Formic acid	Acetic acid	l Ethylene glycol

an Inertsil ODS-4 (5 µm, 250 mm × 4.6 mm ID) column and diode array detector (DAD) was employed. The eluent consisted of 0.1% *o*-phosphoric acid (solvent A) and acetonitrile (solvent B), with a flow rate of 0.8 mL min^{-1} and a run time of 45 min and detection at 220 nm. Gallic acid was identified by comparing its retention time with that of an external commercial standard.

In total, 243 measurements were conducted to perform statistical analyses and optimization. Table 2 presents the Box-Behnken experimental design matrix with corresponding gallic acid concentrations (GA ppm). The coded values (−1, 0, +1) represent the standardized levels of independent variables (A–F), corresponding to low, central, and high factor levels respectively.

Results and discussion

Conventional extraction

Following Soxhlet extraction, the gallic acid content was quantified by HPLC. As shown in Table 3, methanol:water (95:5 v/v) consistently yielded the highest gallic acid levels across all samples, whereas acetone:water (90:10 v/v) produced approximately half those values. However, the lowest concentration (0.018 mg g^{-1} dw) was observed in the S2 (boiled) samples, indicating degradation or leaching of phenolics during boiling. As hydrolysable tannins such as gallic and ellagic acids are water soluble, gallic acid may have been lost when boiling the S2 samples with tap water.

Vasconcelos *et al.* extracted gallic acid from chestnut shells of two different cultivars using 70% MeOH at 70 °C, reporting concentrations between 0.14 and 0.36 mg g^{-1} fresh weight.[23] As fresh samples were used, the values are lower than those in the present study. In another study, extraction of chestnut shells with water at 80 °C for 10 min and at 220 °C for 30 min reduced gallic acid content from 1.36 mg g^{-1} dw to 0.54 mg g^{-1} dw.[24] Otles and Selek analyzed phenolic compounds in chestnut fruits from various regions of Türkiye using 80% MeOH.[25] The Bartin chestnut fruit had the lowest total phenolic content, with a gallic acid concentration of 0.137 mg g^{-1}. These findings indicate that the fruit contains less gallic acid than the shell.

Statistical analysis and optimization

Analysis of variance

A natural logarithmic transformation was applied to the dependent variable, gallic acid concentration (ppm), to satisfy the normality criterion and to meet the requirements for parametric analysis. Following this transformation, an analysis of variance (ANOVA) was performed to determine the statistical significance of the independent variables and their interactions.

Table 4 presents the results of the variance analysis for the parameters and their pairwise interactions. All linear terms were found to be statistically significant ($P < 0.05$), indicating that each independent variable had a meaningful influence on gallic acid concentration. The coefficient of determination ($R^2 = 88.17\%$) suggested a good fit between the experimental data and the values predicted by the model. The *F*-values indicate the relative contribution of each independent variable to the variation in gallic acid content. Based on these values, the most influential variables, in descending order, were material type > liquid-to-solid ratio > extraction temperature > water content > extraction time > DES type.

Another method to assess model adequacy is by evaluating residual normality. Figure 1 shows residual analysis graphs for the logarithmically transformed data. The residuals conform to a normal distribution without skewness or outliers. The residuals versus fit and order plots also indicate random distribution, constant variance, and absence of systematic patterns.

Regression analysis

Table 5 presents the regression coefficients for the linear model established for gallic acid. All independent variables included in the model were statistically significant ($P < 0.05$). Extraction time, extraction temperature, water content, and liquid-to-solid ratio exhibited statistically significant effects on the gallic acid yield, confirming their strong influence on the extraction process. Among the categorical variables, both DES type and material type showed statistically significant differences when compared to their respective reference categories, which were Formic Acid for DES type and Control sample for material type.

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Table 2. Box–Behnken experimental design model and gallic acid values (GA ppm).

No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA
1	−1	−1	0	0	1	1	1.686	62	0	0	1	1	3	1	1.356	123	0	−1	1	0	2	2	14.282	184	0	1	0	−1	1	3	27.540
2	1	−1	0	0	1	1	3.748	63	−1	0	0	−1	3	1	4.93	124	0	1	1	0	2	2	24.947	185	0	−1	0	1	1	3	5.799
3	−1	1	0	0	1	1	2.928	64	1	0	0	−1	3	1	6.55	125	−1	0	−1	0	2	2	3.628	186	0	1	0	1	1	3	9.717
4	1	1	0	0	1	1	7.455	65	−1	0	0	1	3	1	1.706	126	1	0	−1	0	2	2	8.556	187	0	0	0	0	1	3	9.289
5	0	0	−1	−1	1	1	5.888	66	1	0	0	1	3	1	2.201	127	−1	0	1	0	2	2	7.951	188	0	0	0	0	1	3	9.761
6	0	0	1	−1	1	1	9.831	67	0	−1	−1	0	3	1	1.516	128	1	0	1	0	2	2	12.899	189	0	0	0	0	1	3	7.243
7	0	0	−1	1	1	1	2.039	68	0	1	−1	0	3	1	2.23	129	0	−1	0	−1	2	2	26.350	190	−1	−1	0	0	2	3	8.688
8	0	0	1	1	1	1	3.082	69	0	−1	1	0	3	1	3.52	130	0	1	0	−1	2	2	52.537	191	1	−1	0	0	2	3	8.426
9	−1	0	0	−1	1	1	7.16	70	0	1	1	0	3	1	6.037	131	0	−1	0	1	2	2	3.942	192	−1	1	0	0	2	3	24.827
10	1	0	0	−1	1	1	6.506	71	−1	0	−1	0	3	1	2.074	132	0	1	0	1	2	2	17.080	193	1	1	0	0	2	3	40.433
11	−1	0	0	1	1	1	1.608	72	1	0	−1	0	3	1	2.529	133	0	0	0	0	2	2	10.392	194	0	0	−1	−1	2	3	19.468
12	1	0	0	1	1	1	3.694	73	−1	0	1	0	3	1	2.852	134	0	0	0	0	2	2	10.518	195	0	0	1	−1	2	3	56.799
13	0	−1	−1	0	1	1	1.818	74	1	0	1	0	3	1	2.827	135	0	0	0	0	2	2	7.155	196	0	0	−1	1	2	3	12.478
14	0	1	−1	0	1	1	5.052	75	0	−1	0	−1	3	1	5.047	136	−1	−1	0	0	3	2	0.475	197	0	0	1	1	2	3	19.052
15	0	−1	1	0	1	1	4.494	76	0	1	0	−1	3	1	12.867	137	1	−1	0	0	3	2	0.979	198	−1	0	0	−1	2	3	61.882
16	0	1	1	0	1	1	6.318	77	0	−1	0	1	3	1	1.423	138	−1	1	0	0	3	2	0.576	199	1	0	0	−1	2	3	62.075
17	−1	0	−1	0	1	1	0.865	78	0	1	0	1	3	1	4.636	139	1	1	0	0	3	2	1.446	200	−1	0	0	1	2	3	8.225
18	1	0	−1	0	1	1	3.854	79	0	0	0	0	3	1	3.078	140	0	0	−1	−1	3	2	1.901	201	1	0	0	1	2	3	16.441
19	−1	0	1	0	1	1	3.764	80	0	0	0	0	3	1	3.684	141	0	0	1	−1	3	2	10.290	202	0	−1	−1	0	2	3	9.407
20	1	0	1	0	1	1	4.653	81	0	0	0	0	3	1	3.526	142	0	0	−1	1	3	2	0.655	203	0	1	−1	0	2	3	34.904
21	0	−1	0	−1	1	1	6.957	82	−1	−1	0	0	1	2	1.028	143	0	0	1	1	3	2	1.675	204	0	−1	1	0	2	3	16.952
22	0	1	0	−1	1	1	11.319	83	1	−1	0	0	1	2	0.997	144	−1	0	0	−1	3	2	6.451	205	0	1	1	0	2	3	33.974
23	0	−1	0	1	1	1	2.381	84	−1	1	0	0	1	2	4.367	145	1	0	0	−1	3	2	8.486	206	−1	0	−1	0	2	3	59.113
24	0	1	0	1	1	1	4.336	85	1	1	0	0	1	2	8.791	146	−1	0	0	1	3	2	0.918	207	1	0	−1	0	2	3	24.645
25	0	0	0	0	1	1	4.114	86	0	0	−1	−1	1	2	6.355	147	1	0	0	1	3	2	3.037	208	−1	0	1	0	2	3	26.241
26	0	0	0	0	1	1	4.091	87	0	0	1	−1	1	2	10.409	148	0	−1	−1	0	3	2	0.717	209	1	0	1	0	2	3	24.154
27	0	0	0	0	1	1	3.787	88	0	0	−1	1	1	2	1.756	149	0	1	−1	0	3	2	1.337	210	0	−1	0	−1	2	3	29.251
28	−1	−1	0	0	2	1	6.432	89	0	0	1	1	1	2	2.468	150	0	−1	1	0	3	2	2.764	211	0	1	0	−1	2	3	115.382
29	1	−1	0	0	2	1	16.402	90	−1	0	0	−1	1	2	2.163	151	0	1	1	0	3	2	1.395	212	0	−1	0	1	2	3	8.829
30	−1	1	0	0	2	1	8.532	91	1	0	0	−1	1	2	7.817	152	−1	0	−1	0	3	2	0.807	213	0	1	0	1	2	3	28.890
31	1	1	0	0	2	1	20.342	92	−1	0	0	1	1	2	0.65	153	1	0	−1	0	3	2	1.569	214	0	0	0	0	2	3	32.605
32	0	0	−1	−1	2	1	37.072	93	1	0	0	1	1	2	2.499	154	−1	0	1	0	3	2	1.899	215	0	0	0	0	2	3	33.809
33	0	0	1	−1	2	1	38.412	94	0	−1	−1	0	1	2	1.372	155	1	0	1	0	3	2	4.841	216	0	0	0	0	2	3	32.503
34	0	0	−1	1	2	1	6.68	95	0	1	−1	0	1	2	9.71	156	0	−1	0	−1	3	2	6.263	217	−1	−1	0	0	3	3	0.365
35	0	0	1	1	2	1	11.246	96	0	−1	1	0	1	2	2.463	157	0	1	0	−1	3	2	7.081	218	1	−1	0	0	3	3	2.927

(Continues)

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Table 2. (Continued)

No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA	No	A	B	C	D	E	F	GA
36	−1	0	0	−1	2	1	37.786	97	0	1	1	0	1	2	10.215	158	0	−1	0	1	3	2	1.313	219	−1	1	0	0	3	3	1.657
37	1	0	0	−1	2	1	39.423	98	−1	0	−1	0	1	2	0.182	159	0	1	0	1	3	2	0.709	220	1	1	0	0	3	3	2.944
38	−1	0	0	1	2	1	11.38	99	1	0	−1	0	1	2	3.118	160	0	0	0	0	3	2	3.416	221	0	0	−1	−1	3	3	3.728
39	1	0	0	1	2	1	9.685	100	−1	0	1	0	1	2	1.809	161	0	0	0	0	3	2	1.494	222	0	0	1	−1	3	3	5.008
40	0	−1	−1	0	2	1	3.052	101	1	0	1	0	1	2	4.076	162	0	0	0	0	3	2	2.359	223	0	0	−1	1	3	3	0.785
41	0	1	−1	0	2	1	13.702	102	0	−1	0	−1	1	2	3.653	163	−1	−1	0	0	1	3	4.253	224	0	0	1	1	3	3	1.051
42	0	−1	1	0	2	1	17.439	103	0	1	0	−1	1	2	24.936	164	1	−1	0	0	1	3	7.902	225	−1	0	0	−1	3	3	2.481
43	0	1	1	0	2	1	17.943	104	0	−1	0	1	1	2	3.341	165	−1	1	0	0	1	3	9.417	226	1	0	0	−1	3	3	4.415
44	−1	0	−1	0	2	1	1.757	105	0	1	0	1	1	2	6.226	166	1	1	0	0	1	3	15.889	227	−1	0	0	1	3	3	0.823
45	1	0	−1	0	2	1	6.646	106	0	0	0	0	1	2	2.827	167	0	0	−1	−1	1	3	14.434	228	1	0	0	1	3	3	1.902
46	−1	0	1	0	2	1	12.084	107	0	0	0	0	1	2	2.664	168	0	0	1	−1	1	3	22.897	229	0	−1	−1	0	3	3	0.501
47	1	0	1	0	2	1	15.409	108	0	0	0	0	1	2	2.175	169	0	0	−1	1	1	3	7.175	230	0	1	−1	0	3	3	2.845
48	0	−1	0	−1	2	1	10.126	109	−1	−1	0	0	2	2	4.145	170	0	0	1	1	1	3	4.599	231	0	−1	1	0	3	3	2.561
49	0	1	0	−1	2	1	35.873	110	1	−1	0	0	2	2	8.705	171	−1	0	0	−1	1	3	8.679	232	0	1	1	0	3	3	2.278
50	0	−1	0	1	2	1	10.415	111	−1	1	0	0	2	2	30.479	172	1	0	0	−1	1	3	16.534	233	−1	0	−1	0	3	3	0.464
51	0	1	0	1	2	1	9.108	112	1	1	0	0	2	2	24.671	173	−1	0	0	1	1	3	3.411	234	1	0	−1	0	3	3	0.641
52	0	0	0	0	2	1	13.588	113	0	0	−1	−1	2	2	12.007	174	1	0	0	1	1	3	8.551	235	−1	0	1	0	3	3	1.212
53	0	0	0	0	2	1	18.357	114	0	0	1	−1	2	2	23.215	175	0	−1	−1	0	1	3	7.524	236	1	0	1	0	3	3	3.641
54	0	0	0	0	2	1	12.409	115	0	0	−1	1	2	2	3.811	176	0	1	−1	0	1	3	14.653	237	0	−1	0	−1	3	3	7.494
55	−1	−1	0	0	3	1	0.94	116	0	0	1	1	2	2	5.440	177	0	−1	1	0	1	3	7.288	238	0	1	0	−1	3	3	7.472
56	1	−1	0	0	3	1	0.645	117	−1	0	0	−1	2	2	20.514	178	0	1	1	0	1	3	12.722	239	0	−1	0	1	3	3	1.781
57	−1	1	0	0	3	1	2.465	118	1	0	0	−1	2	2	26.517	179	−1	0	−1	0	1	3	3.782	240	0	1	0	1	3	3	0.403
58	1	1	0	0	3	1	5.892	119	−1	0	0	1	2	2	3.823	180	1	0	−1	0	1	3	10.097	241	0	0	0	0	3	3	1.419
59	0	0	−1	−1	3	1	4.095	120	1	0	0	1	2	2	4.896	181	−1	0	1	0	1	3	7.953	242	0	0	0	0	3	3	1.736
60	0	0	1	−1	3	1	8.57	121	0	−1	−1	0	2	2	3.525	182	1	0	1	0	1	3	12.965	243	0	0	0	0	3	3	1.485
61	0	0	−1	1	3	1	0.289	122	0	1	−1	0	2	2	33.135	183	0	−1	0	−1	1	3	15.930								

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Table 3. Gallic acid yield after the conventional extraction.

Sample	Solvent type	GA (ppm)	Yield (mg g^{-1} dw)
S1	Acetone:water	2.80	0.168
	Methanol:water	5.70	0.342
S2	Acetone:water	0.30	0.018
	Methanol:water	0.80	0.048
S3	Acetone:water	14.0	0.840
	Methanol:water	30.3	1.818

Note: dw: dry weight.

Optimum conditions obtained with the BBD

Figure 2 presents the main effects of each experimental factor on the mean amount of gallic acid. Among these, extraction temperature shows a clearly positive relationship, where increasing the temperature from 30 to 70 °C led to a notable increase in gallic acid yield. This trend is likely due to enhanced solubility and diffusion of target compounds at elevated temperatures. In contrast, the liquid-to-solid ratio exhibited a negative relationship, indicating that higher solvent volumes diluted the sample, thus reducing gallic acid concentration.

Water content had a modest positive trend up to 20% but appeared to plateau beyond that, suggesting a saturation point where further addition of water did not improve extraction significantly. Similarly, extraction time had a mild upward effect up to 40 min, after which the increase was marginal, possibly indicating that equilibrium was reached.

Among material types, baked samples resulted in the highest gallic acid concentration whereas boiled materials yielded the lowest, suggesting possible degradation or leaching of gallic acid during boiling. For the DES types, ethylene glycol provided the most favorable results, whereas acetic acid and formic acid had relatively smaller effects, possibly due to their polarity or pH behavior affecting compound stability.

The variables that maximized the value of gallic acid were also identified, and the optimum gallic acid value was noted. The experimental conditions yielding the best results in the established model were determined as: extraction time 39 min; extraction temperature 70 °C; water 18 mL; liquid-to-solid ratio: 10:1; material: baked; DES type: choline chloride:ethylene glycol (Fig. 3). Under these conditions, the amount of gallic acid was estimated to be 116 ppm (logarithmic value: 4.75). The desirability function (d) was found to be 1, indicating excellent agreement between the predicted and experimental values. Using the same model to predict gallic acid yield under the optimal experimental conditions (extraction time: 35 min; temperature: 70 °C; water content: 20 mL; liquid-to-solid ratio: 10; material: baked; DES: choline chloride:ethylene glycol), the predicted value was 115.07 ppm with a desirability of 0.99 – closely matching the experimental results.

Table 4. Analysis of variance results.

Source	DF	Adj SS	Adj MS	*F*	*P*
Model	38	297.379	7.8258	40.01	0.000
Linear	8	261.377	32.6722	167.05	0.000
A	1	9.822	9.8217	50.22	0.000
B	1	17.315	17.3154	88.53	0.000
C	1	10.528	10.5277	53.83	0.000
D	1	45.669	45.6690	233.50	0.000
E	2	162.803	81.4013	416.19	0.000
F	2	15.241	7.6204	38.96	0.000
Square	4	9.610	2.4026	12.28	0.000
AA	1	2.546	2.5465	13.02	0.000
BB	1	0.102	0.1015	0.52	0.472
CC	1	0.850	0.8499	4.35	0.038
DD	1	2.948	2.9478	15.07	0.000
Two-way interaction	26	26.391	1.0150	5.19	0.000
AB	1	0.001	0.0012	0.01	0.938
AC	1	0.367	0.3666	1.87	0.172
AD	1	0.233	0.2327	1.19	0.277
AE	2	1.261	0.6304	3.22	0.042
AF	2	0.444	0.2220	1.13	0.323
BC	1	1.825	1.8245	9.33	0.003
BD	1	0.445	0.4452	2.28	0.133
BE	2	1.794	0.8972	4.59	0.011
BF	2	0.417	0.2083	1.06	0.347
CD	1	0.068	0.0685	0.35	0.555
CE	2	0.693	0.3465	1.77	0.173
CF	2	0.839	0.4197	2.15	0.120
DE	2	1.194	0.5969	3.05	0.049
DF	2	0.149	0.0746	0.38	0.683
EF	4	16.662	4.1654	21.30	0.000
Error	204	39.899	0.1956		
Lack-of-fit	186	39.242	0.2110	5.78	0.000
Pure error	18	0.657	0.0365		
Total	242	337.278			

Abbreviations: Adj MS, adjusted mean squares; Adj SS, adjusted sums of squares; DF, degree of freedom.

Effect of the DES-UAE method

Ultrasound-assisted extraction is a green extraction technique that employs ultrasonic waves to create pressure variations, leading to the formation and collapse of cavitation bubbles.

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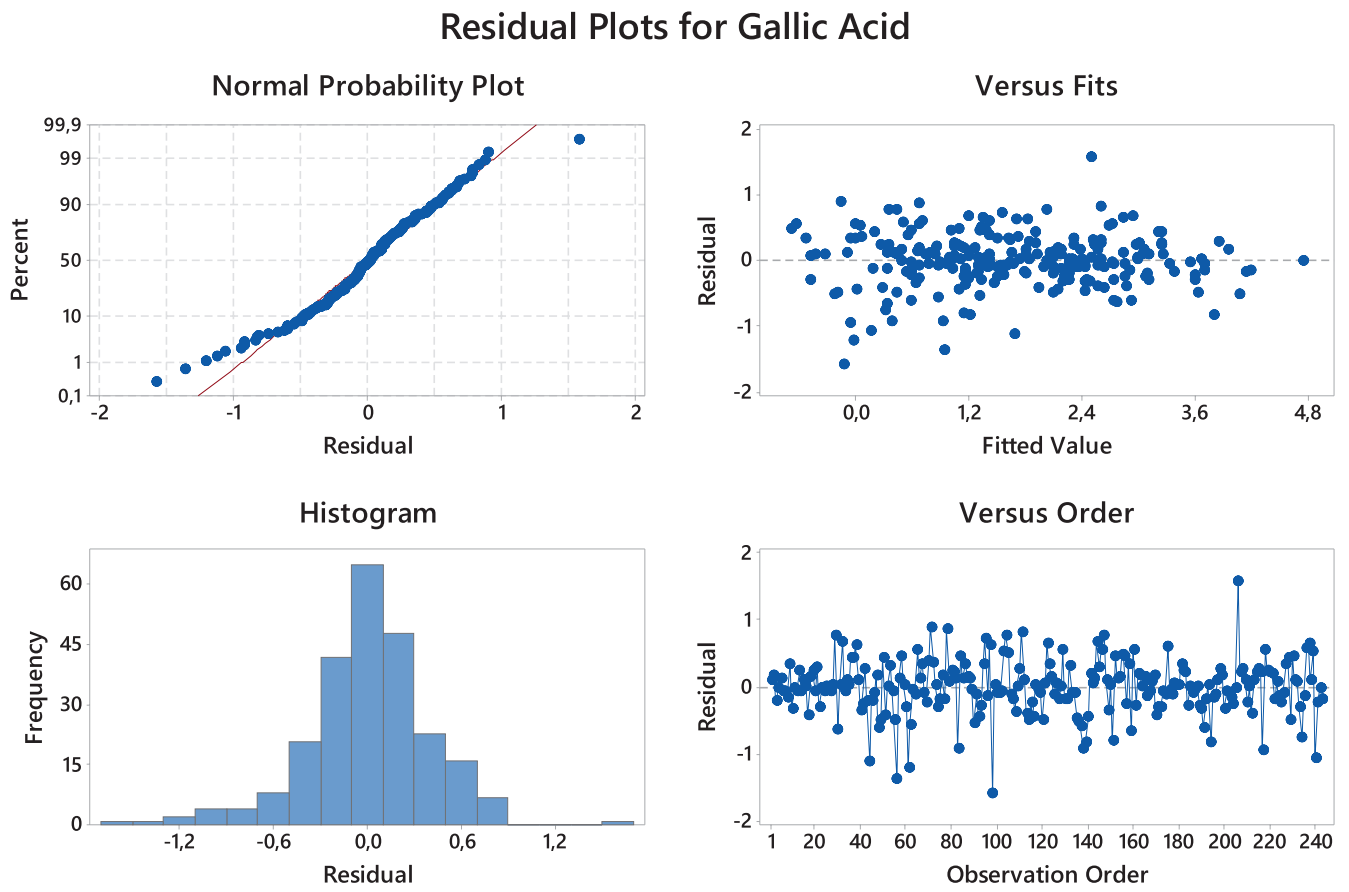

Figure 1. Residual analysis graphs for gallic acid.

Table 5. Linear regression model coefficients for gallic acid.

Term	Coefficients	Standard errors (SEs) of coefficients	*T*	*P*
Constant	0.796	0.235	3.38	0.001
Extraction time (A)	0.01206	0.00219	5.50	0.000
Extraction temperature (B)	0.02002	0.00274	7.31	0.000
Water (C)	0.03122	0.00548	5.70	0.000
Liquid/solid ratio (D)	−0.06503	0.00548	−11.87	0.000
DES (E)				
Acetic acid	−0.2604	0.0895	−2.91	0.004
Ethyl glycol	0.3508	0.0895	3.92	0.000
Material (F)				
Baked	1.1531	0.0895	12.89	0.000
Boiled	−0.8438	0.0895	−9.43	0.000

Note: The regression models established to predict the values of gallic acid is provided in Table 6. *T*, *t*-value (test statistic for regression coefficients, indicating the strength of each variable's effect).

Table 6. Gallic acid prediction models (logarithmic values).

DES	Material	Prediction model
Formic acid	Control sample	Gallic acid = 0.796 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Formic acid	Baked	Gallic acid = 1.949 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Formic acid	Boiled	Gallic acid = −0.048 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Acetic acid	Control sample	Gallic acid = 0.536 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Acetic acid	Baked	Gallic acid = 1.689 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Acetic acid	Boiled	Gallic acid = −0.308 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Ethylene glycol	Control sample	Gallic acid = 1.147 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Ethylene glycol	Baked	Gallic acid = 2.300 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D
Ethylene glycol	Boiled	Gallic acid = 0.303 + 0.01206 A + 0.02002 B + 0.03122 C − 0.06503 D

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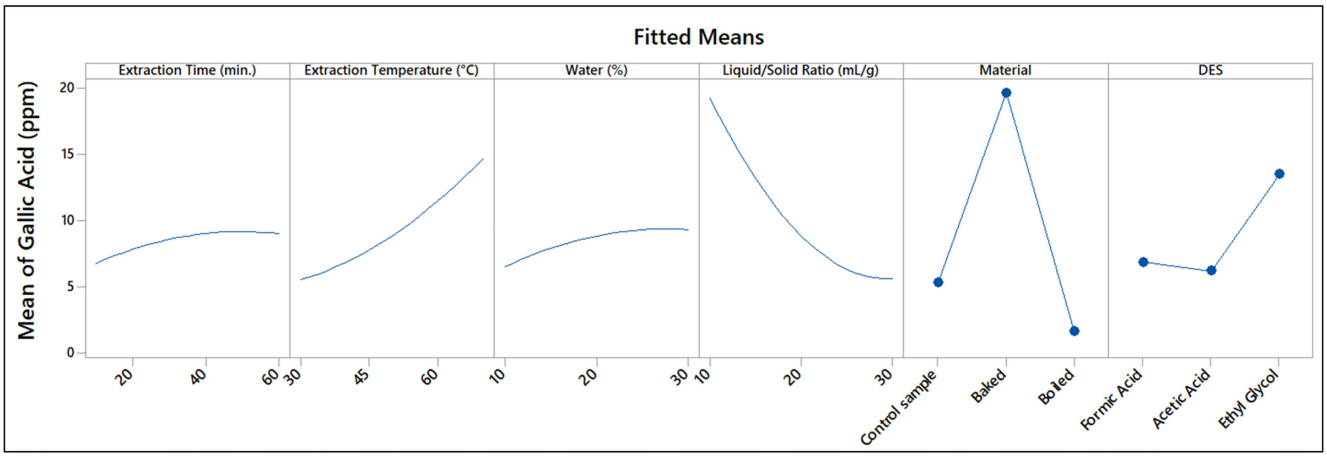

Figure 2. Main effect plot for gallic acid.

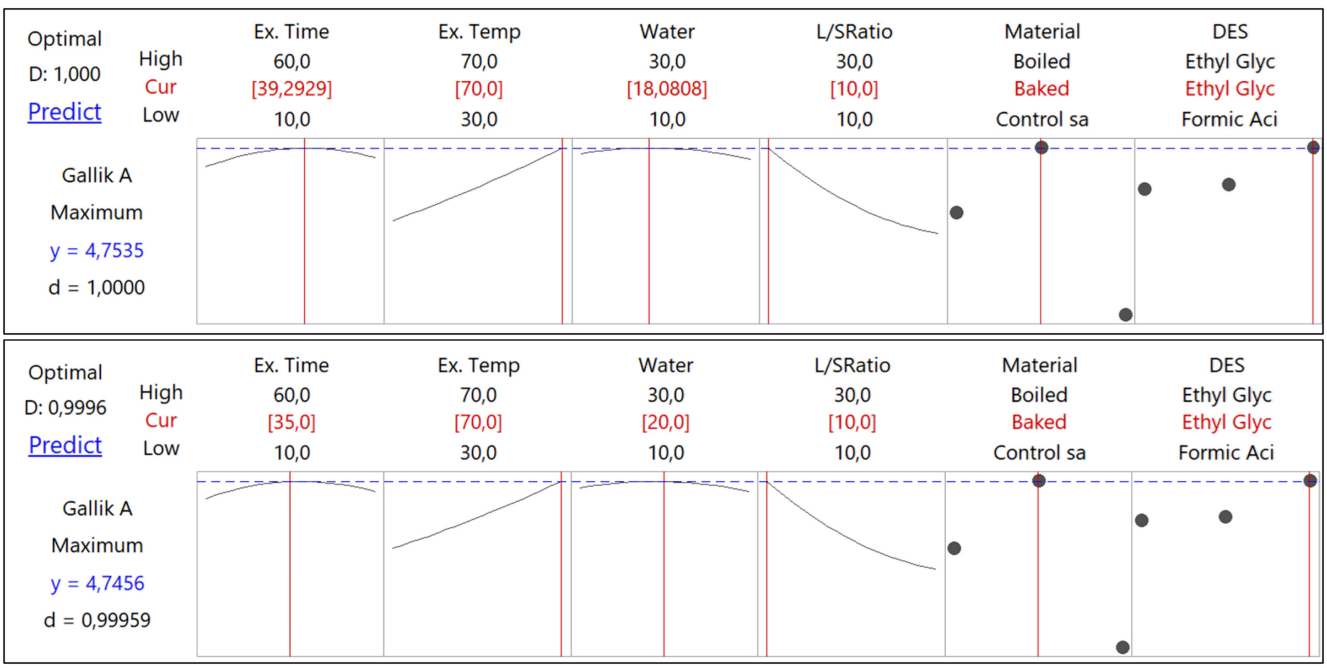

Figure 3. Optimization and prediction graph for factors and levels.

This process disrupts cell structures and enhances mass transfer between the solvent and sample. Ultrasound-assisted extraction using DES improves extraction efficiency and reduces processing time. The mechanism can be summarized in four stages: (1) ultrasonic agitation, generating high-frequency mechanical motion within the DES medium; (2) cell disruption, releasing intracellular compounds; (3) cavitation effects, producing micro-jets and shock waves that facilitate phenolic release; and (4) enhanced solubility and mass transfer, increasing extraction efficiency significantly.[26]

In this study, DES-UAE proved highly effective for extracting gallic acid from chestnut shells. Among 243 experimental runs, samples no. 206 (1.182 mg g^{-1}) and no. 211 (1.154 mg g^{-1}) yielded the highest gallic acid concentrations (Figs. 4 and 5). Optimal conditions included baked chestnut shell material, a choline chloride:ethylene glycol (1:2) DES, extraction at 70 °C for 35 min, 20% water content, and a 10:1 liquid-to-solid ratio. These results confirm the potential of DES-based ultrasound-assisted extraction as a sustainable and efficient method, offering both environmental and yield advantages for the valorization of agroindustrial byproducts.

In addition to the DES-based UAE method, conventional Soxhlet extraction was carried out using methanol and

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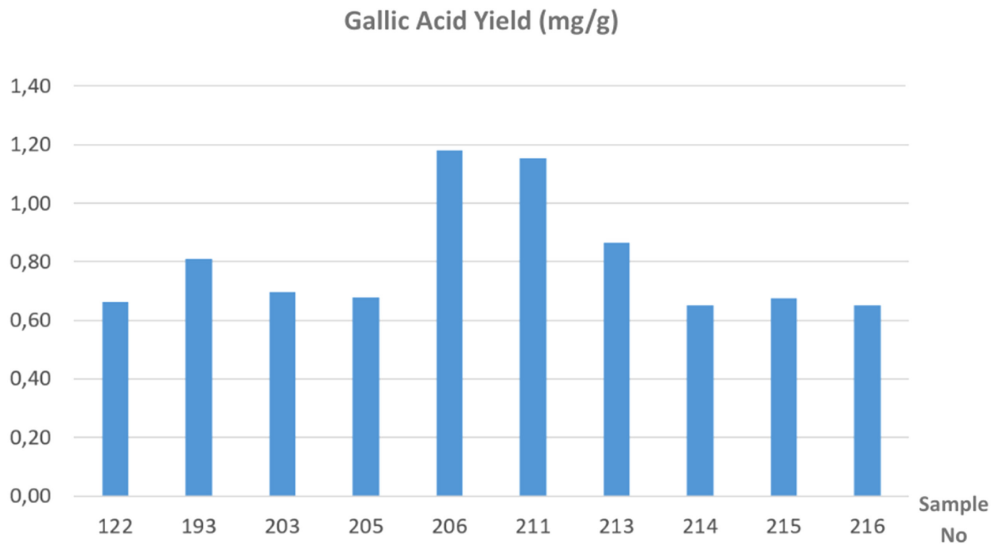

Figure 4. Yield of gallic acid in some chestnut shells with DES-UAE method.

acetone on three chestnut shell samples (S1: untreated, S2: boiled, S3: baked). The highest gallic acid yield with Soxhlet extraction was 1.818 mg g^{-1}, obtained with methanol on baked material (S3). Although this yield slightly exceeded that of the best-performing DES-UAE condition (1.182 mg g^{-1} at 70 °C, 35 min, 20% water, choline chloride:ethylene glycol DES), it required 5 g of sample and 300 mL of solvent over an extended extraction time. By contrast, DES-UAE achieved comparable yields with only 1 g of material and up to 10 mL of solvent, under milder and more environmentally friendly conditions. These results underscore the efficiency and sustainability of DES as green alternatives to conventional organic solvents for natural product extraction. In conclusion, the DES-UAE method reduces extraction costs and energy consumption.

Effect of DES type

Deep eutectic solvent solutions vary in density, pH, and viscosity depending on the specific HBD and HBA components, which in turn influence their penetration and extraction efficiency. The polarity of a DES also impacts its extraction performance significantly.[27] In this study, the use of choline chloride: ethylene glycol (1:2) resulted in the highest gallic acid yield, whereas choline chloride:acetic acid (1:2) produced the lowest.

The inner shell of the chestnut was treated in a reactor at 65 °C for 24 h using various ChCl-based DESs composed of polyols, acids, and sugars. Among them, choline chloride:oxalic acid (1:1) yielded the highest polyphenol content. In accordance with the Kamlet–Taft parameters, DES with higher hydrogen bond donating ability (α) followed the order acids > sugars > polyols, which closely correlated with polyphenol extraction efficiency. Polyol-based DES required the addition of water to reduce viscosity. The type of DES not only influenced the total phenolic yield but also affected the diversity of extracted compounds.[28]

Effect of water amount

Another important factor in the extraction of phenolic compounds with DES solvent is the quantity of water used. Water can be added to the DES system directly or it can form in the system unintentionally. Adding water reduces viscosity, thereby enhancing mass transfer and improving extraction efficiency. High DES viscosity hinders the extraction of biologically active compounds such as polyphenols due to their low mass transport efficiency. It also alters key properties such as the polarity, density, and conductivity of DESs. Changes in polarity can increase the solubility of target compounds when the polarity of the DES matches that of the analyte, facilitating extraction. However, polarity alone does not determine extraction success, as other molecular interactions also influence outcomes. Water can serve as a cosolvent to optimize DES properties but excessive amounts may weaken or even disrupt the DES structure entirely, compromising its effectiveness.

The addition of water not only lowers the viscosity but also reduces the extraction cost.[28,29] According to the literature, the water content varies between 20% and 80% and the optimum amount of water is 20%. Increasing the amount of water negatively affects the extraction as it breaks the hydrogen bond in the DES structure.[26] In this study three different proportions of water (10%, 20%, 30%) were used.

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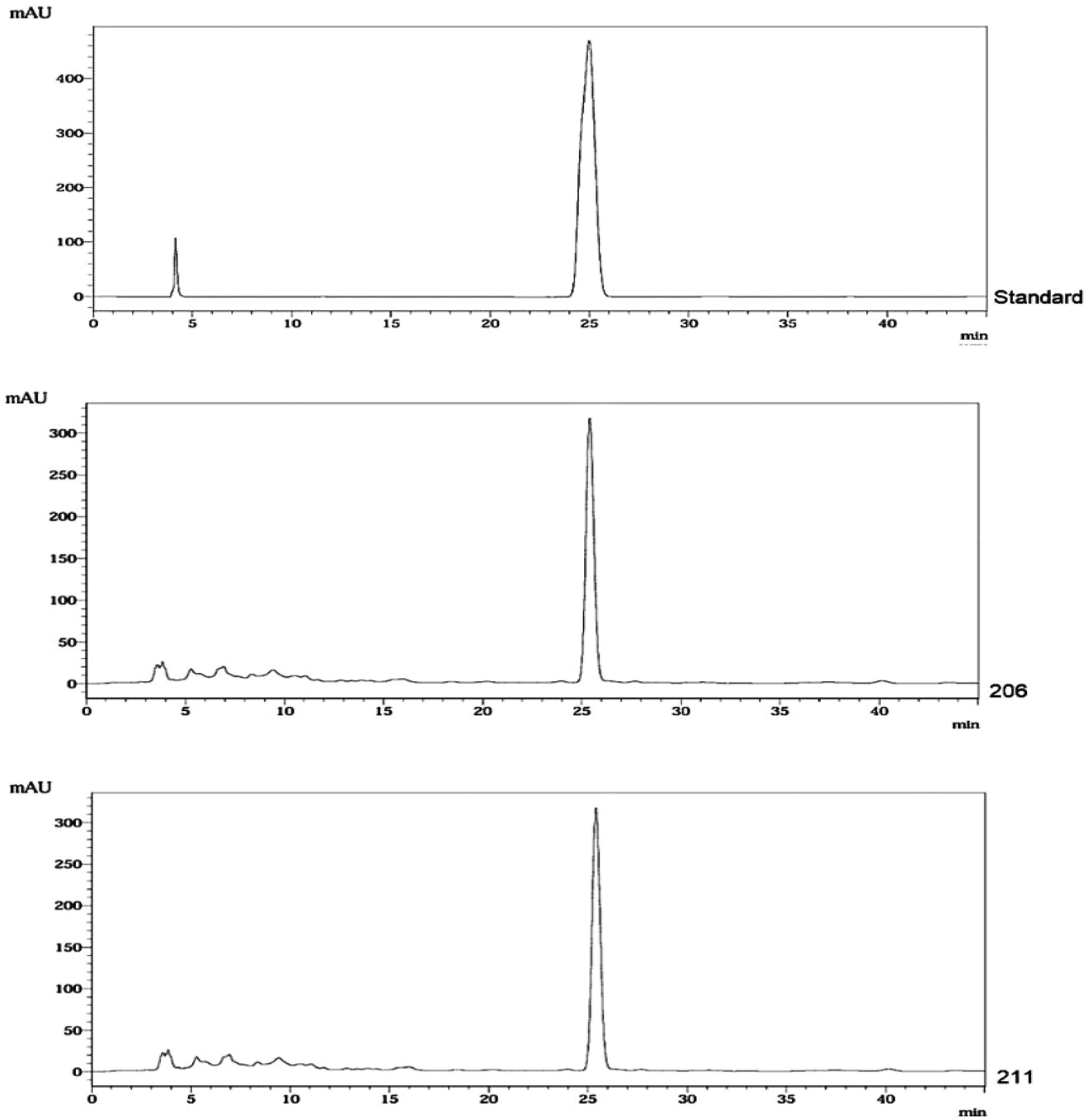

Figure 5. High-performance liquid chromatography chromatograms of gallic acid (standard), Sample 206, Sample 211.

The lowest amount of gallic acid was obtained by using 10% water. High efficiency was obtained by adding 20% water as mentioned above.

Effect of the liquid-to-solid ratio

The liquid-to-solid ratio is a key parameter in DES-based extraction as it affects mass transfer, solubility, and diffusion of target compounds. A higher solvent-to-sample ratio increases the concentration gradient and reduces surface saturation, improving extraction efficiency. However, excessive solvent use can compromise sustainability due to greater demands for solvent recovery. In contrast, a lower ratio may hinder extraction, particularly with rigid plant matrices or compounds with limited diffusivity and solubility. Thus, optimizing this ratio is critical to achieving a balance

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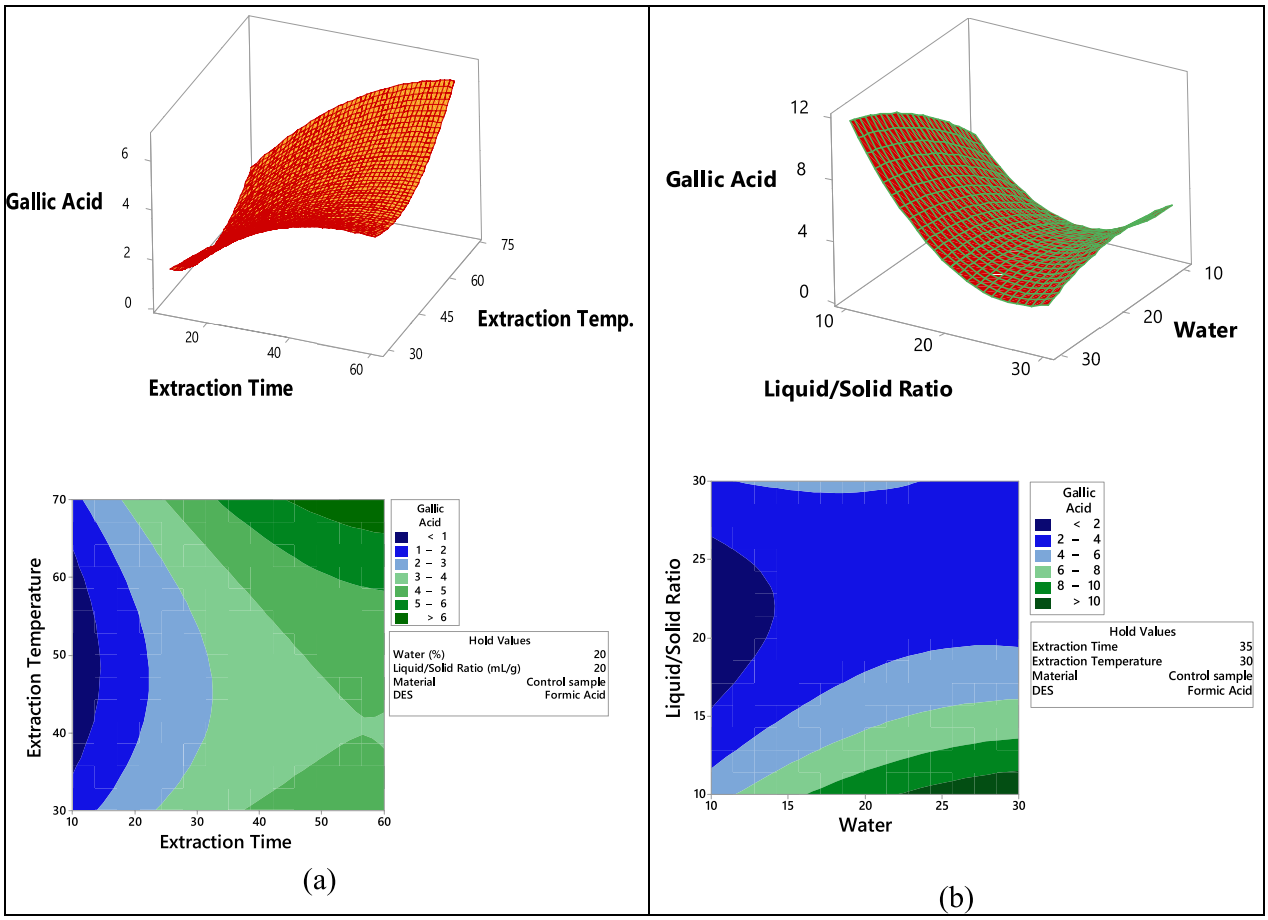

Figure 6. Surface and contour plots showing the effects of (a) extraction temperature and time, and (b) water amount and solid-to-liquid ratio on gallic acid yield.

between yield, efficiency, and environmental impact in green extraction systems employing deep eutectic solvents.[26] In practice, a 20:1 liquid-to-solid ratio is commonly used.[27]

In this study 1 g of sample was treated in an ultrasound bath with 10, 20, and 30 mL DES solution. According to the results, an increase in the liquid-to-solid ratio decreased the gallic acid yield. Optimum conditions were obtained with 1 g of solid and 10 mL of DES solution. The use of a low liquid-to-solid ratio and a large amount of water affected gallic acid yield positively.

Effect of extraction temperature and time

Temperature affects extraction time directly. A long extraction time increases costs whereas a short extraction time causes the target substance to decompose insufficiently. Increasing the temperature causes the molecules to move faster and the solvent to penetrate faster. According to the literature for phenolic compounds the most suitable temperatures for the application of DES solutions are between 25–60 °C and the extraction time between 20 min and 2 h.[30] In this study, the amount of gallic acid increased with the extraction time and temperature. Optimum extraction temperature and time for gallic acid from chestnut shell were found to be 70 °C and 35 min.

The observed increase in gallic acid extraction at higher temperatures and extended extraction times is attributable to improved mass transfer. Elevated temperatures reduce solvent viscosity and enhance molecular motion, facilitating the diffusion of phenolic compounds from the plant matrix into the DES medium. This is supported by the surface and contour plots in Fig. 6, which show a proportional rise in gallic acid content with increasing temperature and time. These trends indicate more efficient desorption and dissolution of target compounds under thermodynamically favorable conditions.

Ultrasound-assisted extraction further amplifies this effect through cavitation, in which high-energy bubbles collapse near the plant surface, generating microjets that disrupt the cellular architecture. This mechanical impact improves

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solvent penetration and accelerates the release of intracellular compounds such as gallic acid.

Moreover, sample preparation was found to influence extraction efficiency significantly, as evidenced by the higher gallic acid content obtained from baked samples. The baking process likely reduces moisture content, increases porosity, and facilitates partial breakdown of the cell-wall structure, all of which contribute to more efficient solvent access and compound solubilization. These effects are reflected indirectly in the optimization plots (Fig. 6), where baked materials combined with high temperatures and low liquid to solid ratios show maximum extraction yields. In contrast, boiling likely causes partial leaching or degradation of hydrolyzable tannins, leading to lower gallic acid recovery.

Conclusion

The valorization of chestnut shell as a sustainable source of gallic acid has significant potential in various industries and aligns with environmental and economic goals. This study confirmed the efficiency of DES-based UAE, particularly using choline chloride:ethylene glycol, for extracting phenolic compounds. The optimization process revealed that baked material and specific DES types enhanced extraction yields, with temperature, time, and water content playing pivotal roles. These findings advocate for the use of DES-based methodologies as green alternatives to conventional solvents, paving the way for innovative, eco-friendly solutions in natural product extraction. The study also underscores the need for further exploration of DES combinations and their applications across diverse industrial sectors.

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