

APPLICATION OF DEEP EUTECTIC SOLVENTS IN EXTRACTION OF BIOACTIVE COMPOUNDS FROM FOOD INDUSTRY BYPRODUCT - COCOA BEAN SHELL

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INTRODUCTION

Cocoa bean shell (CBS), a by-product of the chocolate industry, aside from its basic composition, contains some biologically active compounds, like methylxantines (theobromine, caffeine, theophylline) and some phenolic compounds. These valuable biologically active compounds can be extracted utilizing different extraction techniques and used for different purposes. In continuation of our work on green extraction technologies, we applied deep eutectic solvents (DES) as well as microwave assisted extraction (MAE) in extraction of different compounds from CBS. Two extraction methods were compared, DES extraction with stirring and microwave induced DES extraction (DES-MAE).

MATERIALS AND METHODS

CBS material (Fig. 1) was obtained from chocolate factory Kandit d.o.o. Osijek, Croatia, in the summer of 2017 after the roasting process. After grounding, CBS was extracted using 16 different choline chloride based DESs at 50 °C during 60 minutes, with 10% and 50% water addition (Table 1). The screening showed that DES mixture choline chloride:oxalic acid was the most suitable for the extraction (Table 2). For a comparison, a DES extraction with stirring (Table 3) was compared to the DES-MAE (Fig. 2) of CBS (Table 4) considering gallic acid, theobromine, catechin, caffeine, caffeoic acid and epicatechin. An experimental design and optimisation was performed using response surface methodology (RSM), which included 17 runs of extraction. Antioxidant activity (DPPH) was also determined for the obtained extracts (Table 3 and 4). Identification and quantification of bioactive compounds was performed by high performance liquid chromatography with diode array detection (HPLC/DAD) (Fig. 3).

Table 2. Screening results for different DESs at different % of water, at constant time of 60 min and temperature 50 °C

DES	% H ₂ O	Gallic acid (mg/g)	Theobromin (mg/g)	Catechin (mg/g)	Caffeine (mg/g)	Caffeic acid (mg/g)	Epicatechin (mg/g)	% DPPH
ChCl:AA	10	0.009	3.020	0.052	0.775	0.024	0.031	33.582
	50	0.016	3.003	0.062	0.776	0.029	0.029	44.741
ChCl:BD	10	0.000	2.282	0.000	0.571	0.023	0.051	34.280
	50	0.000	3.639	0.063	0.868	0.034	0.028	53.401
ChCl:EG	10	0.008	3.019	0.057	0.766	0.025	0.019	50.521
	50	0.002	3.133	0.055	0.715	0.028	0.022	52.944
ChCl:Fru	10	0.000	2.062	0.051	0.560	0.026	0.039	48.911
	50	0.000	3.414	0.095	0.752	0.037	0.031	53.067
ChCl:Gly	10	0.000	2.786	0.064	0.734	0.031	0.027	30.41
	50	0.000	3.380	0.062	0.763	0.036	0.033	55.00
ChCl:Glu	10	0.00	2.086	0.048	0.546	0.024	0.034	27.31
	50	0.009	2.413	0.000	0.657	0.031	0.039	52.50
ChCl:MA	10	0.000	1.017	0.00	0.207	0.011	0.000	42.00
	50	0.000	2.655	0.00	0.639	0.030	0.066	50.32
ChCl:Xy	10	0.000	1.362	0.000	0.326	0.016	0.033	23.63
	50	0.000	3.052	0.074	0.684	0.029	0.029	24.08
ChCl:LeA	10	0.000	3.382	0.000	0.916	0.034	0.057	43.44
ChCl:CA	50	0.000	3.032	0.000	0.706	0.027	0.071	45.18
ChCl:Mal	10	0.000	2.773	0.000	0.701	0.023	0.055	42.65
	50	0.000	3.600	0.000	0.865	0.031	0.114	48.04
ChCl:LA	10	0.000	3.145	0.000	0.790	0.036	0.059	39.65
	50	0.000	2.854	0.000	0.665	0.026	0.063	51.52
ChCl:OA	10	0.000	0.620	0.000	0.156	0.000	0.000	64.32
	50	0.000	3.605	0.000	0.909	0.019	0.104	66.31
ChCl:Sor	50	0.000	3.008	0.062	0.635	0.040	0.110	32.15
ChCl:U	10	0.000	2.329	0.000	0.665	0.028	0.022	31.15
	50	0.000	3.613	0.069	0.848	0.037	0.011	50.46

Table 3. Determined active compounds and antioxidant activity in extracts obtained by DES mixture ChCl:OA at different % of water (experiments performed with stirring)

RUN	T (°C)	t (min)	% H ₂ O	Gallic acid (mg/g)	Theobromin (mg/g)	Catechin (mg/g)	Caffeine (mg/g)	Caffeic acid (mg/g)	Epicatechin (mg/g)	% DPPH
1	90	15	30	0.000	4.682	0.042	1.330	0.000	0.064	74.81
2	60	10	30	traces	4.185	0.056	1.436	0.000	0.077	61.09
3	60	10	30	traces	4.241	0.054	1.400	0.000	0.069	51.26
4	90	10	10	traces	3.102	0.045	1.389	0.022	0.034	55.35
5	60	15	50	traces	4.537	0.046	1.524	0.040	0.033	42.02
6	90	5	30	traces	4.564	0.051	1.397	0.058	0.027	48.25
7	60	5	10	traces	2.145	0.035	0.681	0.056	0.030	46.79
8	60	10	30	traces	3.943	0.053	1.243	0.047	0.067	37.55
9	60	10	30	traces	4.010	0.056	1.208	0.040	0.066	33.46
10	60	10	30	traces	4.119	0.053	1.182	0.031	0.061	40.18
11	60	5	50	traces	4.024	0.051	1.252	0.064	0.017	24.03
12	60	15	10	traces	4.010	0.032	0.871	0.098	0.015	31.13
13	30	10	10	traces	2.324	0.031	0.710	0.045	0.011	37.16
14	90	10	50	traces	4.285	0.034	1.289	0.104	0.005	31.42
15	30	15	30	traces	2.630	0.051	1.185	0.073	0.000	24.22
16	30	5	30	0.000	3.195	0.054	1.084	0.043	0.000	30.35
17	30	10	50	0.000	2.877	0.053	1.032	0.047	0.000	35.80

Table 4. Determined active compounds and antioxidant activity in extracts obtained by DES mixture ChCl:OA at different % of water (experiments performed under microwaves)

RUN	T (°C)	t (min)	% H ₂ O	Gallic acid (mg/g)	Theobromin (mg/g)	Catechin (mg/g)	Caffeine (mg/g)	Caffeic acid (mg/g)	Epicatechin (mg/g)	% DPPH
1	90	15	30	0.463	4.754	0.050	1.311	0.120	0.059	32.315
2	60	10	30	0.103	5.004	0.047	1.599	0.103	0.000	55.444
3	60	10	30	0.101	4.912	0.040	1.412	0.083	0.000	55.315
4	90	10	10	0.000	3.348	0.066	0.969	0.098	0.000	48.238
5	60	15	50	0.221	4.823	0.056	1.590	0.128	0.017	48.597
6	90	5	30	0.304	4.765	0.067	1.531	0.067	0.137	41.290
7	60	5	10	0.043	2.502	0.043	0.792	0.047	0.000	24.392
8	60	10	30	0.103	4.941	0.050	1.585	0.096	0.000	43.803
9	60	10	30	0.110</						