

# ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPONENTS FROM COCOA SHELL

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## Introduction

Large quantities of solid waste are created during the processing of raw materials from plants in the food industry every year, and their storage, processing or management present a serious ecological and economic problem. Cocoa shell is only one of the potentially valuable by-products that can be successfully used in the production of extracts that are rich in bioactive components. The aim of this work was to recover some bioactive components from cocoa shell using green innovative technique, ultrasound-assisted extraction.

## Ultrasound-assisted extraction (UAE)

An ultrasound bath with temperature, frequency (37 kHz), ultrasonic power and time control (Elma, Elmasonic P 70 H, Elma Schmidbauer GmbH, Gottlieb-Daimler, Singen, Germany) was used for the UAE experiments. 1.0 g of grounded cocoa shell was mixed with water as a solvent in a 50 mL flask. During the experiments, the temperature varied at 40, 60 and 80 °C, the time varied at 30, 60 and 90 min, solvent-solid ratio 10, 30 and 50 mL/g and ultrasonic power varied at 30, 50, 70 %. Flasks were always positioned at the same distance from the transducer, and no additional agitation was applied. After extraction, crude extracts were immediately filtered through the filter paper. Extracts were collected in glass flasks and stored at 4°C until the HPLC analysis.

## Results

Table 1. Bioactive compounds detected in cocoa shell extracts obtained by HPLC/DAD

RUN	T (°C)	t (min)	Solvent-solid ratio (ml/g)	Power (%)	Gallic acid (mg/kg)	Theobromine (mg/kg)	Coffeine (mg/kg)	Caffeic acid (mg/kg)
1	40	60	30	30	503.77	2076.62	275.81	-
2	60	90	30	70	516.50	2695.60	410.00	255.21
3	40	60	10	50	903.94	3767.00	486.31	-
4	40	90	30	50	621.84	2492.52	360.27	-
5	60	60	30	50	368.15	2121.74	294.55	274.22
6	40	60	30	70	641.59	3119.95	443.69	397.72
7	60	90	10	50	967.96	3871.71	497.19	-
8	80	60	30	70	737.98	4097.64	580.03	447.29
9	60	60	30	50	807.25	4545.08	618.32	527.23
10	60	30	30	70	749.15	3424.06	485.69	443.40
11	40	60	50	50	1197.16	3915.81	569.55	-
12	80	90	30	50	726.82	3725.19	536.51	431.01
13	60	60	30	50	569.50	3610.18	508.26	434.08
14	60	60	50	30	1076.51	5195.34	725.77	-
15	60	60	30	50	769.27	4133.56	588.76	-
16	60	60	50	70	1406.90	5431.12	784.92	-
17	60	30	10	50	865.69	3641.59	433.30	-
18	40	30	30	50	605.46	2254.99	314.67	-
19	60	30	30	30	583.83	3324.93	485.67	392.45
20	60	90	30	30	468.79	3418.58	471.79	380.69
21	80	30	30	50	519.98	2402.08	357.60	-
22	60	90	50	50	1249.31	3600.43	586.69	-
23	60	60	30	50	785.30	3456.36	537.37	-
24	80	60	50	50	1079.90	4127.71	677.86	-
25	80	60	30	30	586.09	2568.00	400.26	-
26	60	60	10	30	853.68	3193.14	439.40	-
27	60	30	50	50	1118.16	4297.28	645.62	-
28	80	60	10	50	687.34	3234.07	420.86	-

## Material

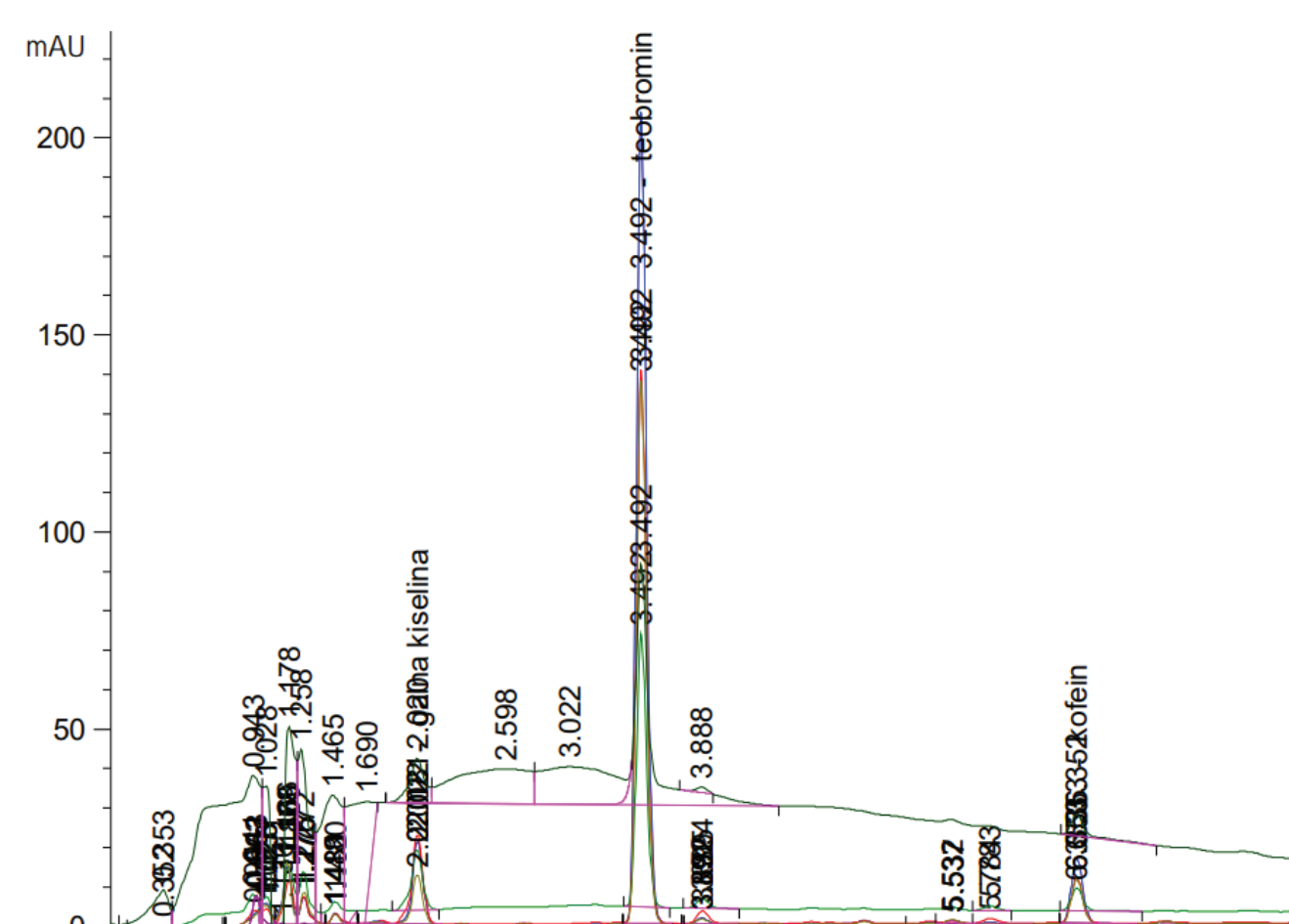
The cocoa shell was obtained from chocolate industry "Kandit d.o.o." Osijek (Croatia). Country of origin of obtained cocoa was Gana and Ivory Coast. All solvents used for the extraction procedures were of analytical grade and purchased from J.T. Baker (PA, USA). Theobromine standard was purchased from Sigma-Aldrich, as well as gallic acid and caffeic acid, while caffeine standard was purchased from Dr. Ehrenstorfer. Before each extraction, cocoa shell was grounded using laboratory mill.

## High Performance Liquid Chromatography (HPLC)

The sample extracts were filtered through 0.2 µm PTFE filter and placed in apparatus for the chromatographic analysis. The measurement was done on HPLC Infinity 1290 Agilent Technologies (USA) instrument with diode array detector (DAD). Injection volume of the sample was 20µL while wavelength was set to 273 nm. The separation was carried out at room temperature using an Agilent Eclipse C<sub>18</sub> column, 4.6 x 250 mm x 5µm. The quantitative analysis was performed with the external standardization by measuring the peak areas and were integrated automatically using the Agilent HPLC Data Analysis software program based on calibration of five standards. The analysis was performed with mobile phase containing water: acetonitrile (80:20 v/v) by isocratic elution with a flow rate of 1,0 ml min<sup>-1</sup>. The run time was set to 10 min. The analysis was conducted in three replications.

## Conclusion

Significant amounts of specific bioactive components from cocoa shell obtained with one of the newest green technologies today, ultrasound-assisted extraction, have shown that this by-product could be used in further food, pharmaceutical, or cosmetic production.



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