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**NEW Installation Research Projects:** *"Application of innovative techniques of the extraction of bioactive components from by-products of plant origin"* (2018-2023)

Principal Investigator: Stela Jokić (Budget: **1.607.708,72 HRK**)











- Food industry waste growing problem today
- Byproducts contain valuable bioactive components

• Could be a raw material in other productions

(food, cosmetics, pharmaceutical..)





- IN THE PRODUCTION OF COCOA AND CHOCOLATE: COCOA SHELL
- The use:
  - feed for livestock
  - fertilizer
  - Fuel
  - Source of dietary fiber





**Components of cocoa shell** 

- Methylxantines (caffeine, theobromine, theophylline)
- Phenolic components (catechin, epicatechin, epigalocatechin, gallic acid, vanillin, caffeic acid, chlorogenic acid...)
- 5- Hydroxymethylfurfural
- Fatty acid composition similar to cocoa butter (higher nutritional value considering double higher linolenic amount than cocoa butter)







#### **GREEN EXTRACTION TEHNIQUES** with numerous advantages





Examples: Supercritical fluid extraction (SFE)
Ultrasonic assisted extraction (UAE)
Cold atmospheric plasma assisted extraction (CAPAE)
Natural deep eutectic solvent extraction (NADES)





















#### Cold atmospheric plasma assisted extraction (CAPAE)







#### Natural deep eutectic solvent (NADES)













#### MATERIAL AND METHODS

 Cocoa shell - Kandit d.o.o. factory, Osijek, Croatia (country of origin Ghana, Ivory Coast)
- 4 different green extraction techniques
-Soxhlet extraction was done for the comparison





#### **EXTRACTION CONDITIONS:**

SFE	p/ bar	Temp /°C	t/min	Flow/kgCO2/h
	200	40	20	2
	500	60	50	2

	t/min	Frequency/Hz	Temp/°C	Solvent used
UAE	30	35	Room, 40, 60	Water
				50% aqueous ethanol solution
				70% aqueous ethanol solution
				96% aqueous ethanol solution



#### EXTRACTION CONDITIONS:

	Frequency/Hz t/mi		in Exti		action solvent	
CAPAE	70				H2O	
	100 30					
NADES	Solvent mix: cholin-chloride:oxalic acid		T/°C		t/ min	
	0% H2O					
	25% H2O		room		60, 180, 360	
	50% H2O					



HPLC **theobromine** and **caffeine** DETERMINATION:

Agilent Technologies 1290 Infinity: autosampler, quaternary pump, DAD Injection volume: 20μl Wavelenght: 273nm Column used: Eclipse C<sub>18</sub> (4.6 x 250mm, 5μm) Mobile phase: water:ACN (80:20) Flow rate: 1ml/min



#### **TPC DETERMINATION:**

By Folin-Ciocalteu spectrophotometric method Selecta UV-2005 spectrophotometer Wavelenght: 765nm

#### % DPPH scavening activity DETERMINATION:

Measured the absorbance at 517nm after 30 minutes of the diluted samples – in triplicate









#### **RESULTS Supercritical CO<sub>2</sub> extraction:**

Extraction parameters	Theobromine (mg/g)	Caffeine (mg/g)	Total phenols (mg GAE/g extract)	% DPPH scavenging activity
40°C	5.87	0.54	10.85	15.90
60°C	6.35	0.65	12.36	15.07

*SC-CO*<sub>2</sub> extraction was performed at pressure 300 bar and 30 min.



#### **Ultrasonic assisted extraction:**

Extraction solvent	Extraction	Theobromine		Total phenols	% DPPH
	naramotors		Caffeine (mg/g)	(mg GAE/g	scavenging
	parameters	(1118/8/		extract)	activity
	Room temp.	4.45	0.51	24.95	39.98
H <sub>2</sub> O	40°C	4.53	0.55	17.26	41.13
	60°C	4.96	0.75	15.97	47.06
	Room temp.	2.10	0.91	14.18	62.41
ethanol	40°C	2.14	0.91	14.28	72.24
	60°C	2.50	0.99	15.46	91.87
	Room temp.	0.39	0.82	11.36	47.31
70%-aqueous	40°C	0.43	0.97	14.95	69.67
ethanoi	60°C	0.46	0.88	15.72	70.28
	Room temp.	-	0.04	10.08	5.59
96% ethanol	40°C	-	0.07	12.36	6.36
	60°C	-	0.18	14.69	7.04





**Cold atmospheric plasma assissted extraction :** 

	Theobromine		Total phenols	% DPPH scavenging	
Frequency (Hz)	(mg/g)	Caffeine (mg/g)	(mg GAE/g extract)	activity	
100	4.44	0.54	16.23	32.21	
70	5.61	0.59	17.00	32.05	



#### Natural deep eutectic solvent extraction:

Extraction parameters		Theobromine		Total phenols	% DPPH
solvent	Time/min	(mg/g)	Carreine (mg/g)	(mg GAE/g extract)	activity
	60	0.26	0.02	10.21	82.84
0 % H <sub>2</sub> 0	180	0.35	0.02	12.90	83.25
	360	0.53	0.02	10.59	85.01
25 % H <sub>2</sub> 0	60	3.70	0.51	29.82	84.75
	180	3.51	0.50	31.38	88.08
	360	3.17	0.51	30.04	89.99
50 % H <sub>2</sub> 0	60	3.64	0.54	32.39	82.34
	180	4.05	0.57	37.90	89.35
	360	4.04	0.58	34.83	92.27



All green extractions gave better yields for both components than conventional Soxhlet extraction

Extraction	Theobromine	Caffeine	
type	(mg/g)	(mg/g)	
Soxhlet	0.00061	0.00146	



Comparison of theobromine and caffeine extraction yields obtained by different green extraction techniques









#### CONCLUSION

**Cocoa shell** as a byproduct of the food industry contains valuable bioactive components such as theobromine and coffeine, as well as some phenolic compound (according to literature dana)

#### **Green extraction techniques**

- great substitute for conventional extractions
- could be used in the future to reduce food industry waste



Thanks to sponsors!









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