

# PHENOLICS AND METHYLYXANTHINES PROFILE OF COCOA SHELL AND THE EFFECT OF COLD PLASMA TREATMENT ON THEIR CONTENT

Veronika Barišić, Ivana Flanjak\*, Ivana Križić, Antun Jozinović, Drago Šubarić, Jurislav Babić, Đurđica Ačkar

PTF

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek,  
Franje Kuhača 20, 31 000 Osijek, Croatia



## Introduction

Cocoa shell, a by-product of cocoa industry, is valuable source of dietary fibers, phenolics, methylxanthines and vitamins. Recently, the presence of high-valuable bioactive components in cocoa shell was recognized and the pallet of food products enriched with cocoa shell is increasing. The most abundant bioactive components of cocoa shell are methylxanthines (theobromine and caffeine) and flavanols (catechins, epicatechins and procyanidins). Cold plasma treatment is often used for decontamination of products but also effects food constituents. The aim of this study was to determine bioactive component profiles of cocoa shell and evaluate the effect of cold plasma treatment of their composition and content.

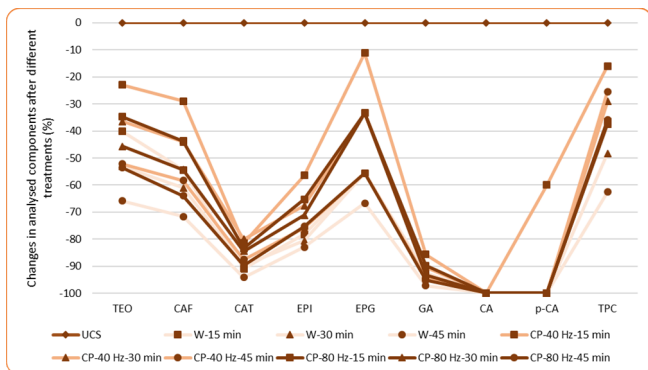
## Materials and methods

Fermented and roasted cocoa shell was obtained from chocolate industry „Kandit d.o.o.” (Osijek, Croatia). Cocoa shell was mixed with water (6 g and 12 g of cocoa shell in 400 ml of water) for 15, 30 and 45 minutes, respectively. Cold plasma water treatment (CP) was performed on cocoa shell using frequencies of 40 and 80 Hz for 15, 30 and 45 minutes. After treatments, cocoa shell was dried and grinded in laboratory mixer. Qualitative and quantitative determination of 6 phenolic components (gallic acid (GA), caffeic acid (CA), p-coumaric acid (p-CA), (+)-catechin (CAT), (-)-epicatechin (EPI) and (-)-epicatechin gallate (EPG)) and 2 methylxanthines (theobromine (TEO) and caffeine (CAF)) was performed by chromatographic method with absorbance detection. Separation of components was performed with gradient elution using methanol:water as mobile phase. Detection of separated components was at 278 nm and quantification with external standard method. Total phenolic content was determined by spectrophotometric Folin-Ciocalteu method.

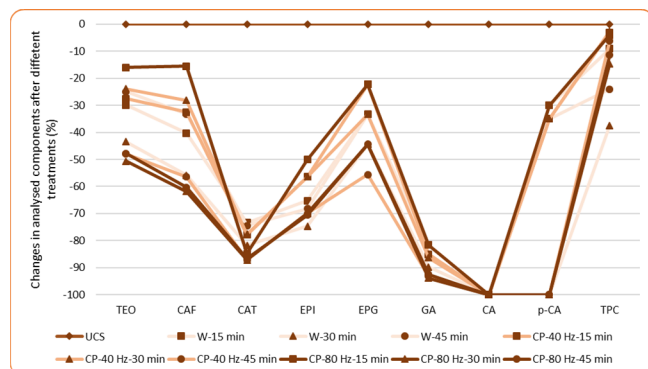
**Table 1** Average values of methylxanthines, polyphenols and total phenolic content in cocoa shell before and after different treatment conditions

	TEO mg/g	CAF mg/g	CAT mg/g	EPI mg/g	EPG mg/g	GA mg/g	CA mg/g	p-CA mg/g	TPC mg GAE/g
Untreated cocoa shell (UCS)	3.906	0.646	0.290	0.165	0.009	0.147	0.004	0.017	28.89
Treatment conditions									
6 g cocoa shell/400 ml water	W-15 min	2.335	0.296	0.026	0.037	0.004	0	0	18.21
	W-30 min	1.832	0.253	0.030	0.033	0.004	0	0	14.93
	W-45 min	1.334	0.184	0.017	0.029	0.003	0	0	10.81
	CP-40 Hz-15 min	3.008	0.462	0.054	0.074	0.008	0.021	0	24.27
	CP-40 Hz-30 min	2.481	0.364	0.058	0.055	0.006	0.014	0	20.48
	CP-40 Hz-45 min	1.868	0.271	0.036	0.041	0.004	0.008	0	21.55
	CP-80 Hz-15 min	2.547	0.366	0.048	0.059	0.006	0.015	0	18.30
	CP-80 Hz-30 min	2.122	0.297	0.045	0.049	0.006	0.010	0	18.08
	CP-80 Hz-45 min	1.813	0.234	0.030	0.042	0.004	0.007	0	18.52
	CP-80 Hz-45 min	2.739	0.388	0.077	0.059	0.006	0.027	0	26.27
12 g cocoa shell/400 ml water	W-15 min	2.209	0.287	0.053	0.043	0.005	0.015	0	18.06
	W-30 min	2.933	0.434	0.074	0.054	0.006	0.021	0	21.95
	W-45 min	2.832	0.439	0.064	0.074	0.007	0.022	0	28.02
	CP-40 Hz-15 min	2.974	0.467	0.065	0.074	0.006	0.020	0	28.15
	CP-40 Hz-30 min	2.032	0.283	0.037	0.051	0.004	0.010	0	27.12
	CP-40 Hz-45 min	3.283	0.549	0.044	0.085	0.007	0.027	0	27.76
	CP-80 Hz-30 min	1.928	0.248	0.037	0.051	0.005	0.009	0	24.67
	CP-80 Hz-45 min	2.036	0.258	0.039	0.050	0.005	0.011	0	25.60
	CP-80 Hz-45 min	2.036	0.258	0.039	0.050	0.005	0.011	0	25.60
	CP-80 Hz-45 min	2.036	0.258	0.039	0.050	0.005	0.011	0	25.60

theobromine (TEO); caffeine (CAF); (+)-catechin (CAT); (-)-epicatechin (EPI); (-)-epicatechin gallate (EPG); gallic acid (GA); caffeic acid (CA); p-coumaric acid (p-CA); total phenolic content (TPC)



**Figure 1** Intensity of changes (%) of analysed bioactive components in cocoa shell (6 g cocoa shell/400 ml water) after different treatment conditions with respect to untreated cocoa shell (UCS)



**Figure 2** Intensity of changes (%) of analysed bioactive components in cocoa shell (12 g cocoa shell/400 ml water) after different treatment conditions with respect to untreated cocoa shell (UCS)

## Results

The results showed that theobromine, caffeine and (+)-catechin are major bioactive components of untreated cocoa shell followed by gallic acid and (-)-epicatechin (Table 1). Untreated cocoa shell has the highest values of methylxanthines, polyphenols and total phenolic content in compare to treated cocoa shells. Although any water treatment decreased the content of bioactive components compared to untreated cocoa shell, the decrease was lower when cold plasma water treatment was applied. Also, intensity of changes of bioactive components was greater in treatments with 6 g of cocoa shell in 400 ml of water (Figure 1) than with 12 g of cocoa shell in 400 ml of water (Figure 2).



## Acknowledgment

This work has been supported by Croatian Science Foundation under the project „Application of cocoa husk in production of chocolate and chocolate-like products” (UIP-2017-05-8709)

