Food and Bioprocess Technology (2018) 11:818–835
https://doi.org/10.1007/s11947-017-2045-6

ORIGINAL PAPER

Pulsed Electric Field Assisted Extraction of Bioactive Compounds from Cocoa Bean Shell and Coffee Silverskin

Letricia Barbosa-Pereira[1] · Alessandro Guglielmetti[1] · Giuseppe Zeppa[1]

Received: 30 June 2017 / Accepted: 12 December 2017 / Published online: 13 January 2018

Abstract

The present study focused on the application of pulsed electric fields (PEF) as an innovative pre-treatment technique to improve the recovery of polyphenols from two food by-products, cocoa bean shell (CBS) and coffee silver skin (CS). The effect of the different operating parameters on the extraction of polyphenols was optimised using the response surface methodology statistical approach. The optimised methodology was compared with conventional extraction and applied to several CBS and CS samples to classify the samples according to origin, variety and industrial treatment. PEF-assisted extraction had higher (approximately 20%) recovery yields of polyphenols and methylxanthines than conventional extraction. Finally, the results highlighted that the composition of bioactive compounds from different extracts of CBS and CS and their antioxidant properties depended on the origin, variety and industrial processing of the raw material. These by-products may be a promising source of natural compounds, with potential applications on food and health sectors.

Keywords Cocoa bean shell · Coffee silverskin · Pulsed electric field · Polyphenols · Antioxidant activity · Methylxanthines

Introduction

Cocoa and coffee beans are important sources of phenolic compounds, such as flavan-3-ols (monomeric epicatechin, catechin, oligomers and proanthocyanidins) and flavonols (quercetin glycosides) in cocoa and chlorogenic acids in coffee (Dorenkott et al. 2014; Patras et al. 2014; Upadhyay et al. 2012). Dietary intake of cocoa and coffee has been demonstrated to positively impact human health due to the antioxidant and free radical scavenging properties of these bioactive compounds (Martín and Ramos 2016; Dorenkott et al. 2014; Butt and Sultan 2011). Additionally, these products contain an important level of methylxanthines, such as theobromine in cocoa and caffeine in cocoa and coffee, which are of pharmacological interest due to their stimulatory and positive effects on the central nervous system as well as on the gastrointestinal, vascular and respiratory systems (Steinberg et al. 2003; Mussatto et al. 2011; Anderson and Smith 2002; Martínez-Pinilla et al. 2015).

In recent years, consumption of cocoa and coffee has increased; consequently, tons of cocoa bean shell (CBS) and coffee silverskin (CS), the most important by-products derived from the roasting process of these beans, are produced every year (Kaplinsky 2004). Although these by-products represent a disposal problem, they might also be an economic source of polyphenolic compounds and methylxanthines, which have great potential in industrial applications as food additives/ingredients or supplements of high nutritional value (Martínez-Pinilla et al. 2015; Murthy and Naidu 2012). Therefore, new strategies for the recovery of high-added value compounds, such as polyphenols and/or methylxanthines from these by-products, could be an interesting, sustainable approach to finding new low-cost ingredients.

Usually, traditional extraction methods are very time-consuming and require high levels of solvents and heating (Wang and Weller 2006). Recently, there has been an increasing demand for novel extraction techniques that are environmentally friendly and energy-efficient to enhance mass transfer processes, improve the extract quality and reduce the extraction time and solvent consumption while avoiding the use of organic solvents. Among these emerging techniques,

✉ Letricia Barbosa-Pereira
letricia.barbosapereira@unito.it

[1] Department of Agriculture, Forest and Food Sciences (DISAFA), University of Turin, Grugliasco, Italy

microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), high pressure extraction (HPE), high-voltage electrical discharges (HVED) and pulsed electric fields (PEF) have shown to be efficient at enhancing the overall yield and selectivity of biomolecules from different vegetal matrices (Azmir et al. 2013; Barba et al. 2015b, d; Misra et al. 2017; Guglielmetti et al. 2017).

Of note, PEF is a non-thermal technique that increases mass transfer due to the permeabilisation of cell membranes induced by electroporation under the effect of electric pulses with a short duration (ranged from nanoseconds to milliseconds) and moderate electric field strength. This technique might accelerate the release of intracellular compounds and increase the extraction rates and yields of different components from vegetal matrices with low energy consumption and low environmental impact. Additionally, PEF-assisted processing might reduce heat-sensitive compounds degradation and facilitate the extract purification (Donsì et al. 2010; Barba et al. 2015c, d; Parniakov et al. 2015a; Wiktor et al. 2015; Puértolas and Barba 2016).

PEF has been used for the recovery of several compounds, such as antioxidants, polyphenols, carotenoids, carbohydrates, proteins and peptides, from different fruits and vegetables and their by-products, such as grapes (Boussetta et al. 2009; Barba et al. 2015a; Rajha et al. 2014; Medina-Meza and Barbosa-Cánovas 2015), blueberry (Bobinaitė et al. 2015), raspberry (Medina-Meza et al. 2013), blackberries (Barba et al. 2015b), microalgae (Parniakov et al. 2015a), sesame cake (Sarkis et al. 2015), brown rice (Quagliariello et al. 2016), papaya peels and papaya seeds (Parniakov et al. 2014, 2015b), mango peels (Parniakov et al. 2016), flaxseed hulls (Boussetta et al. 2014) and orange peel (Luengo et al. 2013).

To the best of our knowledge, no published information is available in the literature related to the PEF effect and optimisation procedure for PEF-assisted extraction of phenolic compounds from cocoa and coffee products or their by-products.

Thus, the main aim of this study was to evaluate the potential advantages of the combined use of PEF pre-treatment with solid-liquid extraction to enhance the yield of bioactive compounds from cocoa and coffee by-products. For this purpose, the parameters of the PEF pre-treatment (electric field intensity, time of treatment and number of cycles) and solid-liquid extraction (% of ethanol and extraction time) were optimised for polyphenol extraction from both matrices using response surface methodology coupled with central composite design. The optimal extraction conditions were also evaluated with several samples of CBS and CS of different varieties, geographical origins and treatments for comparison with those obtained via traditional extractions without PEF pre-treatment.

Materials and Methods

Chemicals

Methanol (≥ 99.9%), formic acid (98–100%), hydrochloric acid (fuming 37%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (97%) (Trolox), 2,2-diphenyl-1-picrylhydrazyl (95%) (DPPH), Folin-Ciocalteu's phenol reagent, sodium carbonate (≥ 99.5%), vanillin (99%), aluminium chloride (99%), sodium nitrite (≥ 99%), (+)-catechin hydrate (> 98%), theobromine (≥ 98.5%) and 5-caffeoylquinic acid were obtained from Sigma-Aldrich (Milano, Italy). Ethanol (≥ 99.9%), sodium hydroxide (1 M), gallic acid, epicatechin and caffeine (≥ 99%) were obtained from Fluka (Milano, Italy). Ultrapure water was prepared in a Milli-Q filter system (Millipore, Milan, Italy).

Samples

Twelve samples of CBS and 12 samples of CS from different origins, varieties and production processes were kindly supplied by local cocoa and coffee manufactures (Table 1). Samples were ground to a powder with 0.3 mm mesh size using an ultra-centrifugal mill Retsch ZM 200 (Retsch Gmbh, Haan, Germany). Samples were stored under vacuum at 4 °C before analysis. The dry mater content of the samples (94–95% for CBS and 96–97% for CBS) was determined using a Gibertini Eurotherm electronic moisture balance (Gibertini Elettronica, Novate Milanese MI, Italy).

Extraction Procedure

Pulsed Electric Field Pre-treatment

The pulsed electric field pre-treatment was performed using a S-P1500 system (Alintel SRL, Pieve di Centro, Italy) with a high-voltage pulsed generator at 12 kV and 100 A. The treatment chamber was a polypropylene cylinder with a diameter of 30 mm, two parallel steel electrodes and a surface area of 7.07 cm^2. Preliminary analysis, including the determination of electrical conductivity using an EC electrode (Jenway 4510 conductivity meter, Stone, Staffordshire, UK), of both matrices indicated that different operational conditions should be established for each matrix to obtain the maximum yield from PEF technology application (data not shown). Then, the distances between the two electrodes were set at 21.8 and 21.3 mm for CBS and CS, respectively, and the volume of the chamber was fixed at 15.40 cm^3 for CBS and 15.05 cm^3 for CS. For each experiment, 0.1 g samples were suspended in 14 mL of water for CBS and 14 mL of an ethanol/water solution (0.7 mL of ethanol and 13.3 mL of water) for CS, which

Table 1 Samples of cocoa bean shell (CBS) and coffee silverskin (CS) classified according to variety, origin and production process

CBS				CS				
Sample code	Variety	Origin	Supplier[a]	Sample code	Variety	Coffee treatment	Roasting	Supplier[a]
VNZ1	*Trinitario*	Venezuela	A	ADS	*Arabica*	Dry	Soft	D
VNZ2	*Criollo* × *Trinitario*	Venezuela	B	ADM	*Arabica*	Dry	Medium	D
CLB1	*Trinitario*	Colombia	A	ADH	*Arabica*	Dry	Hard	D
CLB2	*Trinitario*	Colombia	A	AWS	*Arabica*	Wet	Soft	D
ECD1	*Forastero*	Ecuador	B	AWM	*Arabica*	Wet	Medium	D
ECD2	*Trinitario*	Ecuador	C	AWH	*Arabica*	Wet	Hard	D
TND	*Trinitario*	Trinidad	A	RDS	*Robusta*	Dry	Soft	D
JAV	*Forastero*	Java	A	RDM	*Robusta*	Dry	Medium	D
MEX	*Trinitario*	Mexico	A	RDH	*Robusta*	Dry	Hard	D
HND	*Criollo* × *Trinitario*	Honduras	B	RWS	*Robusta*	Wet	Soft	D
MGC	*Criollo* × *Trinitario*	Madagascar	B	RWM	*Robusta*	Wet	Medium	D
STM	*Forastero*	São Tomé	C	RWH	*Robusta*	Wet	Hard	D

[a] Samples supplied by Guido Gobino S.r.l. (A), Guido Castagna S.r.l. (B), Pastiglie Leone S.r.l. (C) and Torrefazione della Piazza (D)

were loaded into the treatment chamber and subjected to PEF pre-treatments under different working conditions (PEF treatment time, number of pulses and PEF strength) defined by CCD (Table 2). Pulses were delivered at a fixed frequency of 50 Hz.

The temperature and pH were measured before and after PEF pre-treatment with PEF using a portable pH meter Knick Portamess® 913 (Knick, Berlin, Germany). The initial temperature before PEF treatment was Ti = 25 °C and the increase of temperature after treatment was less than 3 °C (see Table 3), while the pH was stable during the treatments (4.88 ± 0.09 for CBS and 5.80 ± 0.09 for CS).

Extraction Procedure

Samples treated with PEF were diluted to 50 mL with the ethanol/water mixtures specified by the CCD to extract polyphenols. Extractions were performed at 25 °C under constant rotatory agitation at 60 rpm using a VDRL 711 orbital shaker (Asal S.r.l., Milan, Italy) for the amount of time defined by CCD. To evaluate the effect of the PEF pre-treatment on the recovery of polyphenols and methylxanthines, untreated samples extracted using the same solid-liquid extraction protocol were used as controls. For this process, 0.1 g of CBS or CS was directly mixed with 50 mL of ethanol at the concentration specified by the CCD. Extractions were performed as previously described at 25 °C under constant rotatory agitation at 60 rpm using a VDRL 711 orbital shaker for the amount of time defined by the CCD. All extracts were centrifuged at 10,400×*g* for 10 min at 4 °C, and the supernatants were then filtered through a 0.22-μm nylon membrane filter. Samples were stored at − 18 °C in the dark before analysis.

Experimental Design

Response surface methodology (RSM) was employed to determine the optimum levels of the PEF treatment time (X_1, range from 5 to 20 μs), number of pulses (X_2, range from 500 to 1000), PEF strength applied (X_3, range from 1.5 to 3 kV cm^{-1} for CBS and from 1.30 to 4.40 kV cm^{-1} for CS), ethanol concentration (X_4, range from 30 to 70% *v*/*v*) and extraction time (X_5, range from 30 to 120 min) to maximise the yield of the total phenolic content (Y_{TPC}). These conditions were selected based on the preliminary experimental results. Each independent variable was coded at five levels, − 1.414, − 1, 0, 1 and + 1.414 (Table 2).

The variables were coded according to the following equation:

$$X_i = \frac{x_i - x_m}{\Delta x} \quad (1)$$

where X_i is the coded value of an independent variable, x_i is the real value of an independent variable, x_m is the mean of the real values of an independent variable and Δx is the step change value.

The response function was Y = mg of GAE (gallic acid equivalent) g^{-1} of CBS and CS. The central composite design (CCD) was arranged to allow for to fit a second-order model and was defined by the Design-Expert® software 9.05 (Stat-Ease, Inc., Minneapolis, MN, USA). The CCD consisted of 53 experiments, including 32 factorial points, 10 star points and 10 replicates at the centre point (Table 3). All experiments were performed in a random order to minimise the effect of unexplained variability in the observed response due to systematic errors.

The extraction yield of the total phenolic content (Y_{TPC}) versus the five variables X_1, X_2, X_3, X_4 and X_5 was evaluated

Table 2 Experimental values and coded levels of the independent variables for central composite design used for cocoa bean shell (CBS) and coffee silverskin (CS)

Independent variables		Symbol	Coded variable levels				
			− 1.414	− 1	0	1	+ 1.414
PEF treatment time (μs)		x_1	5	9	13	16	20
Number of pulses		x_2	500	645	750	855	1000
PEF strength (kV cm^{-1})	CBS	x_3	1.50	1.93	2.25	2.57	3.00
	CS		1.30	2.20	2.85	3.50	4.40
Ethanol concentration (%)		x_4	30	41.6	50	58.4	70
Extraction time (min)		x_5	30	56	75	94	120

using a polynomial second-order model according to the following equation (Eq. 2), which was used in response surface analysis to predict the optimum conditions of the extraction process:

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i X_i + \sum_{i=1}^{5} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{ij} X_i X_j \qquad (2)$$

where Y represents the predicted response (TPC yield) and X_i's are the levels of variables (PEF treatment time, number of pulses, PEF strength, % of ethanol and extraction time). β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for the intercept, linearity, quadratic and interaction, respectively.

Verification of the Model and Application

To test the accuracy of the response surface models, additional PEF-assisted trials were performed using the optimal conditions defined by the model for CBS and CS samples, and the experimental data of the total phenolic content yields were compared to the values predicted by the regression model.

Furthermore, the optimal conditions of PEF-assisted extraction of bioactive compounds were applied to all samples of CBS and CS, as reported in Table 1, and compared to conventional solid-liquid extraction without the PEF pre-treatment. The extraction procedures were performed in triplicate. The extracts obtained under optimal conditions were also analysed for the levels of flavonoids and condensed tannins as well as antioxidant capacity. Finally, the content of specific phenolic compounds and methylxanthines were determined by HPLC.

Analytical Determinations

Total Phenolics

The content of total phenolic compounds (TPC) was determined according to the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965) with some modifications and adapted to a 96-well microplate.

An aliquot (20 μL) of the sample extract was mixed with 100 μL of Folin-Ciocalteu aqueous reagent (10% *v/v*) in the wells of a 96-well microplate. After 3 min, 75 μL of a 7.5% sodium carbonate anhydrous solution was added and the obtained solution was mixed. The solution was allowed to stand for 2 h at 25 °C, and the absorbance was measured at 740 nm against a blank in a spectrophotometric multi-detection microplate reader BioTek Synergy HT (BioTek Instruments, Milan, Italy). All determinations were performed in triplicate. Quantification was performed based on the standard curve of commercial gallic acid (20–100 mg L^{-1}), and the concentration of the total phenolic compounds was expressed as mg of gallic acid equivalents (GAE) g^{-1} of dry weight.

Flavonoids

The content of total flavonoids (TFC) was evaluated according to the colorimetric assay described by Herald et al. (2012). An aliquot (25 μL) of sample extract was mixed in the wells of a 96-well microplate with 100 μL of water and 10 μL of 50 g L^{-1} sodium nitrite. After 5 min, 15 μL of 100 g L^{-1} aluminium chloride were added to the mixture and left to stand for 6 min at 25 °C. Then, 50 μL of 1 M sodium hydroxide and 50 μL of distilled water were added. The plate was shaken for 30 s and the absorbance was measured at 510 nm against a blank of distilled water in a spectrophotometric multi-detection microplate reader BioTek Synergy HT (BioTek Instruments, Milan, Italy). All determinations were performed in triplicate. The calibration curve was prepared with a standard solution of catechin (0–250 mg L^{-1}). The total flavonoid yield was expressed as mg CE g^{-1} of dry weight.

Tannins

The total content of tannins (TTC) was assessed according to the colorimetric assay described by Herald et al. (2014) with some modifications. An aliquot (25 μL) of the sample extract was mixed in the wells of a 96-well microplate with 250 μL of a solution of 4% vanillin reagent in methanol/hydrochloric

Table 3 Central composite design arrangement, temperature variations and observed response values for the PEF-assisted extraction of total phenolic compounds (TPC) from cocoa bean shell (a) CBS and coffee silverskin (b) CS)

Runs	Coded variables					Uncoded variables					ΔT	Response
	X_1	X_2	X_3	X_4	X_5	x_1 Time (μs)	x_2 Number of pulses	x_3 PEF strength (kV cm^{-1})	x_4 Ethanol (%)	x_5 Extraction time (min)	°C	TPC (mg GAE g^{-1} dw)
(a) CBS												
1	1	−1	1	−1	1	16	645	2.57	41.60	94	1.1	31.43
2	1	1	−1	−1	1	16	855	1.93	41.60	94	1.4	30.90
3	−1	1	1	1	1	9	855	2.57	58.40	94	0.0	30.19
4	−1	−1	−1	−1	−1	9	645	1.93	30.00	56	2.0	27.40
5	1	1	−1	1	−1	16	855	1.93	58.40	56	1.7	31.59
6	−1	−1	−1	1	1	9	645	1.93	58.40	94	2.0	28.18
7	0	0	0	0	0	13	750	2.25	50.00	75	1.7	27.62
8	1	−1	1	1	−1	16	645	2.57	58.40	56	1.0	30.74
9	0	0	0	0	0	13	750	2.25	50.00	75	1.6	28.10
10	−1	1	1	−1	−1	9	855	2.57	41.60	56	0.0	27.56
11	0	0	0	0	0	13	750	2.25	50.00	75	1.2	28.41
12	1	−1	−1	−1	1	16	645	1.93	41.60	94	1.4	29.49
13	1	−1	−1	1	−1	16	645	1.93	58.40	56	1.3	32.30
14	1	1	1	−1	1	16	855	2.57	41.60	94	0.5	31.80
15	−1	−1	1	−1	−1	9	645	2.57	41.60	56	0.5	30.30
16	1	1	1	1	−1	16	855	2.57	58.40	56	0.3	26.57
17	0	0	0	0	0	13	750	2.25	50.00	75	1.2	27.40
18	−1	1	−1	−1	−1	9	855	1.93	41.60	56	1.5	30.51
19	−1	−1	1	1	1	9	645	2.57	58.40	94	0.3	28.90
20	−1	1	−1	1	1	9	855	1.93	58.40	94	1.0	31.69
21	−1	1	1	−1	1	9	855	2.57	41.60	94	0.2	32.25
22	1	1	−1	1	1	16	855	1.93	58.40	94	0.7	30.70
23	1	−1	1	1	1	16	645	2.57	58.40	94	0.3	29.61
24	0	0	0	0	0	13	750	2.25	50.00	75	1.2	30.23
25	−1	−1	−1	1	−1	9	645	1.93	58.40	56	1.6	29.77
26	−1	1	1	1	−1	9	855	2.57	58.40	56	0.5	29.13
27	1	−1	1	−1	−1	16	645	2.57	41.60	56	0.3	30.64
28	−1	−1	−1	−1	−1	9	645	1.93	41.60	56	1.3	30.11
29	0	0	0	0	0	13	750	2.25	50.00	75	1.3	28.31
30	1	1	−1	−1	−1	16	855	1.93	41.60	56	0.4	28.59
31	−1	1	−1	−1	1	9	855	1.93	41.60	94	0.9	30.16
32	1	1	1	1	1	16	855	2.57	58.40	94	1.0	29.13
33	−1	−1	1	1	−1	9	645	2.57	58.40	56	1.0	29.56
34	1	1	1	−1	−1	16	855	2.57	41.60	56	0.7	24.93
35	1	−1	−1	−1	−1	16	645	1.93	41.60	56	0.5	29.62
36	0	0	0	0	0	13	750	2.25	50.00	75	1.2	29.09
37	−1	−1	1	−1	1	9	645	2.57	41.60	94	0.9	30.95
38	−1	1	−1	1	−1	9	855	1.93	58.40	56	0.6	28.90
39	0	0	0	0	0	13	750	2.25	50.00	75	1.1	27.39
40	1	-1	-1	1	1	16	645	1.93	58.40	94	0.7	28.77
41	0	0	0	0	0	13	750	2.25	50.00	75	1.8	27.25
42	0	0	1.414	0	0	13	750	3.00	50.00	75	1.0	30.70
43	0	0	0	0	0	13	750	2.25	50.00	75	1.9	25.73
44	0	1.414	0	0	0	13	1000	2.25	50.00	75	0.2	25.29

Table 3 (continued)

Runs	Coded variables					Uncoded variables					ΔT	Response
	X_1	X_2	X_3	X_4	X_5	x_1 Time (μs)	x_2 Number of pulses	x_3 PEF strength (kV cm^{-1})	x_4 Ethanol (%)	x_5 Extraction time (min)	°C	TPC (mg GAE g^{-1} dw)
45	0	0	0	0	1.414	13	750	2.25	50.00	120	0.7	30.10
46	0	0	0	1.414	0	13	750	2.25	70.00	75	0.2	24.94
47	0	− 1.414	0	0	0	13	500	2.25	50.00	75	0.5	25.36
48	1.414	0	0	0	0	20	750	2.25	50.00	75	0.4	31.86
49	0	0	0	0	0	13	750	2.25	50.00	75	1.8	27.44
50	0	0	0	− 1.414	0	13	750	2.25	30.00	75	0.2	27.12
51	0	0	0	0	− 1.414	13	750	2.25	50.00	30	0.3	30.14
52	0	0	− 1.414	0	0	13	750	1.50	50.00	75	0.2	28.50
53	− 1.414	0	0	0	0	5	750	2.25	50.00	75	0.4	31.31
(b) CS												
1	− 1	− 1	− 1	1	1	9	645	2.20	58.40	94	0.5	12.24
2	− 1	− 1	− 1	− 1	− 1	9	645	2.20	41.60	56	0.5	13.04
3	0	0	0	0	0	13	750	2.85	50.00	75	0.8	11.84
4	1	1	0	− 1	1	16	855	2.85	41.60	94	0.5	12.06
5	0	0	0	0	0	13	750	2.85	50.00	75	0.7	12.20
6	1	− 1	1	− 1	1	16	645	3.50	41.60	94	0.1	11.64
7	− 1	1	1	− 1	− 1	9	855	3.50	41.60	56	0.1	11.25
8	− 1	1	1	1	1	9	855	3.50	58.40	94	0.9	12.78
9	1	− 1	1	1	− 1	16	645	3.50	58.40	56	1.9	12.02
10	1	1	− 1	1	− 1	16	855	2.20	58.40	56	1.0	13.59
11	− 1	− 1	1	− 1	− 1	9	645	3.50	41.60	56	0.8	11.55
12	1	− 1	− 1	− 1	1	16	645	2.20	41.60	94	0.7	11.14
13	0	0	0	0	0	13	750	2.85	50.00	75	1.2	11.27
14	0	0	0	0	0	13	750	2.85	50.00	75	1.3	11.41
15	− 1	1	− 1	1	1	9	855	2.20	58.40	94	0.0	13.25
16	− 1	− 1	1	1	1	9	645	3.50	58.40	94	0.8	11.32
17	− 1	1	− 1	− 1	− 1	9	855	2.20	41.60	56	0.2	12.09
18	1	1	1	− 1	1	16	855	3.50	41.60	94	0.1	11.50
19	1	− 1	− 1	1	− 1	16	645	2.20	58.40	56	0.1	12.70
20	1	1	1	1	− 1	16	855	3.50	58.40	56	2.1	12.02
21	1	− 1	1	− 1	− 1	16	645	3.50	41.60	56	1.9	12.17
22	− 1	1	1	1	− 1	9	855	3.50	58.40	56	1.4	12.04
23	− 1	− 1	− 1	1	− 1	9	645	2.20	58.40	56	0.2	11.94
24	1	1	− 1	1	1	16	855	2.20	58.40	94	0.4	13.34
25	1	1	− 1	− 1	− 1	16	855	2.20	41.60	56	0.5	12.06
26	− 1	− 1	− 1	− 1	1	9	645	2.20	41.60	94	0.4	11.60
27	0	0	0	0	0	13	750	2.85	50.00	75	1.1	11.05
28	1	− 1	1	1	1	16	645	3.50	58.40	94	0.2	12.22
29	− 1	1	1	− 1	1	9	855	3.50	41.60	94	0.1	11.82
30	0	0	0	0	0	13	750	2.85	50.00	75	0.7	11.57
31	1	− 1	− 1	− 1	− 1	16	645	2.20	41.60	56	0.2	12.14
32	− 1	1	− 1	1	− 1	9	855	2.20	58.40	56	0.0	12.81
33	− 1	− 1	1	− 1	1	9	645	3.50	41.60	94	0.8	12.50
34	− 1	− 1	1	1	− 1	9	645	3.50	58.40	56	1.3	12.14
35	1	1	1	− 1	− 1	16	855	3.50	41.60	56	2.5	11.14

Table 3 (continued)

Runs	Coded variables					Uncoded variables					ΔT	Response
	X_1	X_2	X_3	X_4	X_5	x_1 Time (μs)	x_2 Number of pulses	x_3 PEF strength (kV cm^{-1})	x_4 Ethanol (%)	x_5 Extraction time (min)	°C	TPC (mg GAE g^{-1} dw)
36	1	1	1	1	1	16	855	3.50	58.40	94	0.5	13.38
37	0	0	0	0	0	13	750	2.85	50.00	75	1.1	11.43
38	− 1	1	− 1	− 1	1	9	855	2.20	41.60	94	0.5	11.32
39	0	0	0	0	0	13	750	2.85	50.00	75	0.7	11.46
40	1	− 1	− 1	1	1	16	645	2.20	58.40	94	0.3	12.14
41	0	0	1.414	0	0	13	750	4.40	50.00	75	1.1	13.10
42	0	0	0	1.414	0	13	750	2.85	70.0	75	0.5	13.72
43	0	0	0	0	0	13	750	2.85	50.00	75	1.0	12.00
44	0	0	0	0	0	13	750	2.85	50.00	75	1.1	12.36
45	0	0	0	0	0	13	750	2.85	50.00	75	0.8	12.17
46	− 1.414	0	0	0	0	5	750	2.85	50.00	75	2.0	13.16
47	0	0	0	− 1.414	0	13	750	2.85	30.0	75	1.2	12.39
48	0	1.414	0	0	0	13	1000	2.85	50.00	75	0.5	12.94
49	1.414	0	0	0	0	20	750	2.85	50.00	75	0.7	12.72
50	0	− 1.414	0	0	0	13	500	2.85	50.00	75	2.5	13.37
51	0	0	− 1.414	0	0	13	750	1.30	50.00	75	1.5	12.94
52	0	0	0	0	− 1.414	13	750	2.85	50.00	30	1.1	11.79
53	0	0	0	0	1.414	13	750	2.85	50.00	120	0.7	12.80

ΔT temperature variation (°C), *GAE* gallic acid equivalent

acid 37% (2:1 *v*/*v*) that was prepared daily. The microplate was incubated for 20 min at 25 °C and the absorbance was read at 500 nm against a blank in a spectrophotometric multi-detection microplate reader BioTek Synergy HT (BioTek Instruments, Milan, Italy). All determinations were performed in triplicate. The calibration curve was prepared with a standard solution of catechin (0–250 mg L^{-1}). The tannin content was expressed as mg of catechin equivalents (CE) g^{-1} of dry weight.

DPPH Assay

The antioxidant activity of CBS and CS extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) radical scavenging method described by von Gadow et al. (1997) with slight modifications.

An aliquot of extract solution (20 μL) was added to 180 μL of a DPPH solution (6×10^{-5} in ethanolic solution 80%) in the wells of a 96-well microplate. The mixture was vigorously shaken and left to stand in the dark for 30 min at 25 °C. The decrease in DPPH absorbance was measured at 517 nm in a spectrophotometric multi-detection microplate reader BioTek Synergy HT (BioTek Instruments, Milan, Italy). All determinations were performed in triplicate. The inhibition percentage (IP) of radical DPPH was calculated according to the following equation:

$$\mathrm{IP}\ (\%) = \frac{(A_0 - A_{30})}{A_0} \times 100 \tag{3}$$

where A_0 is the absorbance at initial time and A_{30} is the absorbance at 30 min.

Trolox was used as a standard at 12.5–300 μM. The radical scavenging activity values (RSA) of each sample were expressed as μmol TE g^{-1} of dry weight.

RP-HPLC-PDA Analysis

Chromatographic analysis was performed with a HPLC-PDA Thermo-Finnigan Spectra System (Thermo-Finnigan, Waltham, USA). The system was equipped with a P2000 binary gradient pump, SCM 1000 degasser, AS 3000 automatic injector and Finnigan Surveyor PDA Plus detector. The ChromQuest software (version 5.0) was used for instrument control as well as data collection and processing.

The compounds were separated on a reverse phase Kinetex Phenyl-Hexyl C18 column (150 × 4.6 mm internal diameter and 5 μm particle size) (Phenomenex, Castel Maggiore, Italy) thermostated at 35 °C. A gradient elution method was applied.

The following solvents constituted the mobile phase: 0.1% formic acid (solvent A) and 100% methanol (solvent B). The elution conditions were as follows: 0–0.5 min, 90% A and 10% B; 0.5–3 min, linear gradient from 10 to 30% B; 3–8 min, 30–35% B; 8.0–11.0 min, 35–40% of B; 11.0–30.0 min, 40–80% of B; and 30.0–31.0 min, 80–10% of B, 31.0–32.0 90% A and 10% of B. The mobile phase flow rate was 1.0 mL min^{-1} and the sample injection volume was 10 μL. Scanning was performed continuously at wavelengths between 200 and 400 nm, and data were acquired at 325 nm for 5-caffeoylquinic acid (5-CQA), 278 nm for epicatechin and 273 nm for caffeine and theobromine. Quantification was assessed using the external linear calibration curves determined under the same conditions with the following correlation coefficients: $R^2 = 0.9993$ for 5-CQA, $R^2 = 0.9998$ for epicatechin, $R^2 = 0.9997$ for theobromine and $R^2 = 0.9960$ for caffeine.

Statistical Analysis

Multiple regression analysis and analysis of variance (ANOVA) of the experimental data were performed using the Design-Expert® 9.05 software (Stat-Ease, Inc., Minneapolis, MN, USA).

The adequacy of each model was determined by evaluating the lack of fit, coefficient of determination (R^2) and coefficient of variation (% CV). The significance of each coefficient was determined using an F test obtained from ANOVA. The regression coefficients were then used to generate response surfaces by assigning central values to three of the five variables and solving the fitted equations as a quadratic equation in the remaining two variables.

The polyphenol and methylxanthine contents of CBS and CS samples determined under optimal conditions were compared by variance analysis with Duncan's post hoc test at the 95% confidence level performed on the Statistica version 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

Results

Optimisation of the PEF-Assisted Extraction Parameters

Model Fitting, Response Surface Analysis and Optimisation for CBS

The total phenolic content (Y_{TPC}) of the CBS extracts obtained from 53 experiments is listed in Table 3 (a). The extracted polyphenol content ranged from 24.93 (experiment run 34) to 32.30 mg GAE g^{-1} dw (experiment run 13). Several controls were performed for all CCD experiments according to the solid-liquid extraction conditions (time and solvent composition). The use of PEF pre-treatment improved the extraction yield of polyphenols up to 34.7% compared with control tests. The highest content of TPC observed in these experiments was significantly higher than that obtained by Arlorio et al. (2005) (18.2 ± 8.4 mg GAE g^{-1} dw) by supercritical CO_2 extraction or that obtained by Martínez et al. (2012) by solid-liquid extraction of CBS using ethanol as the solvent (TPC ranged between 1.02–1.09 mg GAE g^{-1} dw).

The data obtained from the central composite design were fitted to second-order polynomial equations, and the significance of the coefficient of the models was determined by analysis of variance (ANOVA). The coefficients and corresponding p values for each variable are shown in Table 4.

Ten factors were found to be significant ($p < 0.05$) or highly significant ($p < 0.01$) for the CBS model (Table 4). The extraction time (X_5) and quadratic effect of the number of pulses (X_2^2) were significant ($p < 0.05$), while the quadratic terms for the PEF treatment time (X_1^2), PEF strength (X_3^2) and extraction time (X_5^2) were highly significant ($p < 0.01$). The interaction effects between the number of pulses and PEF strength (X_2X_3), PEF number of pulses/extraction time (X_2X_5), and ethanol concentration/extraction time (X_4X_5) were highly significant ($p < 0.01$), while the interaction between the PEF treatment time/number of pulse (X_1X_2) and PEF strength/extraction time (X_3X_5) was significant ($p < 0.05$).

Considering the significant variables, the model obtained by RSM that shows the relationships between the TPC and extraction parameters has a correlation coefficient of $R^2 = 0.7739$ and an $R^2_{Adj.} = 0.7144$, which indicate a satisfactory correlation between the experimental values and those predicted by the equations. The obtained model is expressed by the following quadratic polynomial equation:

$$\begin{aligned} \text{TPC (CBS)} = {} & 52.117 - 0.641x_5 - 0.019x_1x_2 - 0.014x_2x_3 \\ & + 0.046x_2x_5 + 0.041x_3x_5 - 0.037x_4x_5 \\ & + 0.059x_1^2 + 0.013x_2^2 + 1.659x_3^2 \\ & + 0.016x_5^2 \end{aligned} \quad (4)$$

The statistical significance of this reduced regression equation was evaluated by analysis of variance (ANOVA). A p value less than 0.0001 indicates that the model is significant and can be used to optimise the extraction of polyphenols for this by-product. The validity of the model was confirmed using lack of fit testing as reported in Table 4. Analysis of variance for the lack of fit test was not significant ($p > 0.05$), highlighting that the fitting model was adequate to describe the experimental data. The low value of the coefficient of variation (% CV = 3.45) indicated a high degree of precision and good deal of reliability of the experimental values for the model.

Table 4 Analysis of variance (ANOVA) of the second-order polynomial models for the total polyphenolic content of extracts yielded with PEF from cocoa bean shell (CBS) and coffee silverskin (CS)

Source	CBS					CS				
	SS	df	MS	*F* value	*p* value	SS	df	MS	*F* value	*p* value
Model	142.07	20	7.10	7.09	< 0.0001**	16.31	20	0.82	5.84	< 0.0001**
X_1	0.15	1	0.15	0.15	0.6987	0.00641	1	0.00641	0.046	0.8319
X_2	0.26	1	0.26	0.26	0.6148	0.20	1	0.20	1.42	0.2440
X_3	0.00159	1	0.00159	0.00159	0.9685	0.71	1	0.71	5.11	0.0318*
X_4	0.85	1	0.85	0.85	0.3650	4.57	1	4.57	32.74	< 0.0001**
X_5	5.95	1	5.95	5.94	0.0214*	0.091	1	0.091	0.65	0.4259
$X_1 X_2$	5.79	1	5.79	5.78	0.0231*	0.11	1	0.11	0.80	0.3793
$X_1 X_3$	2.66	1	2.66	2.66	0.1141	0.00115	1	0.00115	0.00821	0.9284
$X_1 X_4$	0.76	1	0.76	0.76	0.3921	0.56	1	0.56	3.98	0.0559
$X_1 X_5$	0.19	1	0.19	0.19	0.6699	0.00427	1	0.00427	0.031	0.8625
$X_2 X_3$	10.09	1	10.09	10.07	0.0036**	0.32	1	0.32	2.27	0.1434
$X_2 X_4$	0.34	1	0.34	0.34	0.5621	2.54	1	2.54	18.21	0.0002**
$X_2 X_5$	15.07	1	15.07	15.05	0.0006**	0.90	1	0.90	6.44	0.0170*
$X_3 X_4$	3.89	1	3.89	3.88	0.0587	0.15	1	0.15	1.10	0.3036
$X_3 X_5$	5.72	1	5.72	5.71	0.0238*	1.18	1	1.18	8.44	0.0071**
$X_4 X_5$	11.19	1	11.19	11.17	0.0024**	0.33	1	0.33	2.39	0.1330
X_1^2	41.39	1	41.39	41.32	< 0.0001**	1.06	1	1.06	7.61	0.0101*
X_2^2	4.43	1	4.43	4.42	0.0446*	1.77	1	1.77	12.67	0.0013**
X_3^2	13.86	1	13.86	13.83	0.0009**	1.30	1	1.30	9.33	0.0049**
X_4^2	1.32	1	1.32	1.31	0.2615	1.42	1	1.42	10.16	0.0035**
X_5^2	19.66	1	19.66	19.63	0.0001**	0.023	1	0.023	0.16	0.6885
Residual	28.04	28	1.00			3.91	28	0.14		
Lack of fit	22.38	22	1.02	1.08	0.5050	3.63	23	0.17	3.59	0.0588
Pure error	5.66	6	0.94			0.28	6	0.046		
Cor total	194.49	52				26.02	52			

SS sum of squares, *df* degrees of freedom, *MS* mean square, X_1 PEF treatment time (μs), X_2 number of pulses, X_3 PEF strength (kV cm^{-1}), X_4 concentration of ethanol (%), X_5 extraction time (min)

*$p < 0.05$, **$p < 0.01$

Three-dimensional response surfaces and two-dimensional contour plots were generated by the model for the results of the total polyphenolic content in CBS extracts to evaluate the most significant interactions of variables and their effect in the CCD (Fig. 1a). The plots were obtained depicting two variables within the experimental range, while the other three variables were fixed constant at their respective centre value of the testing ranges. The interaction of number of pulses with the PEF strength (X_2X_3) is shown in Fig. 1a (a). CBS extracts with high amounts of polyphenols can be achieved using either higher values of PEF strength in combination of low values of pulses or using high values of number of pulses with lower values of PEF strength. These results are in agreement with the observations described by others that moderate PEF treatment with relatively low values of PEF strength and the different pulse parameters, are the main variables that govern the efficiency of PEF treatment and their effect was particularly pronounced at room temperature (Luengo et al. 2013; Puértolas and Barba 2016). In the present study, the initial temperature before PEF treatment was $T_i = 25$ °C and the final temperature after electrical treatment T_f never exceeded 27 °C ($\Delta T \leq 2.0$ °C) (see Table 3 (a)). This data highlighted that the use of the present conditions can avoid the decrease on TPC yields and consequently the antioxidant activity values described by Parniakov et al. (2014), after PEF pre-treatment due to the highest increases of temperature ($\Delta T = 15$ °C). In general, the use of high values of PEF strength increases the electroporation and consequently the mass transport phenomena, which result in higher extraction yields, but also the ohmic heating intensity occurs and consequently the occurrence of electrolysis that leads to significant losses in antioxidant activity. Additionally, the increase of yields could be due to the presence of other compounds than polyphenols such as proteins that can be extracted at higher PEF inputs or higher

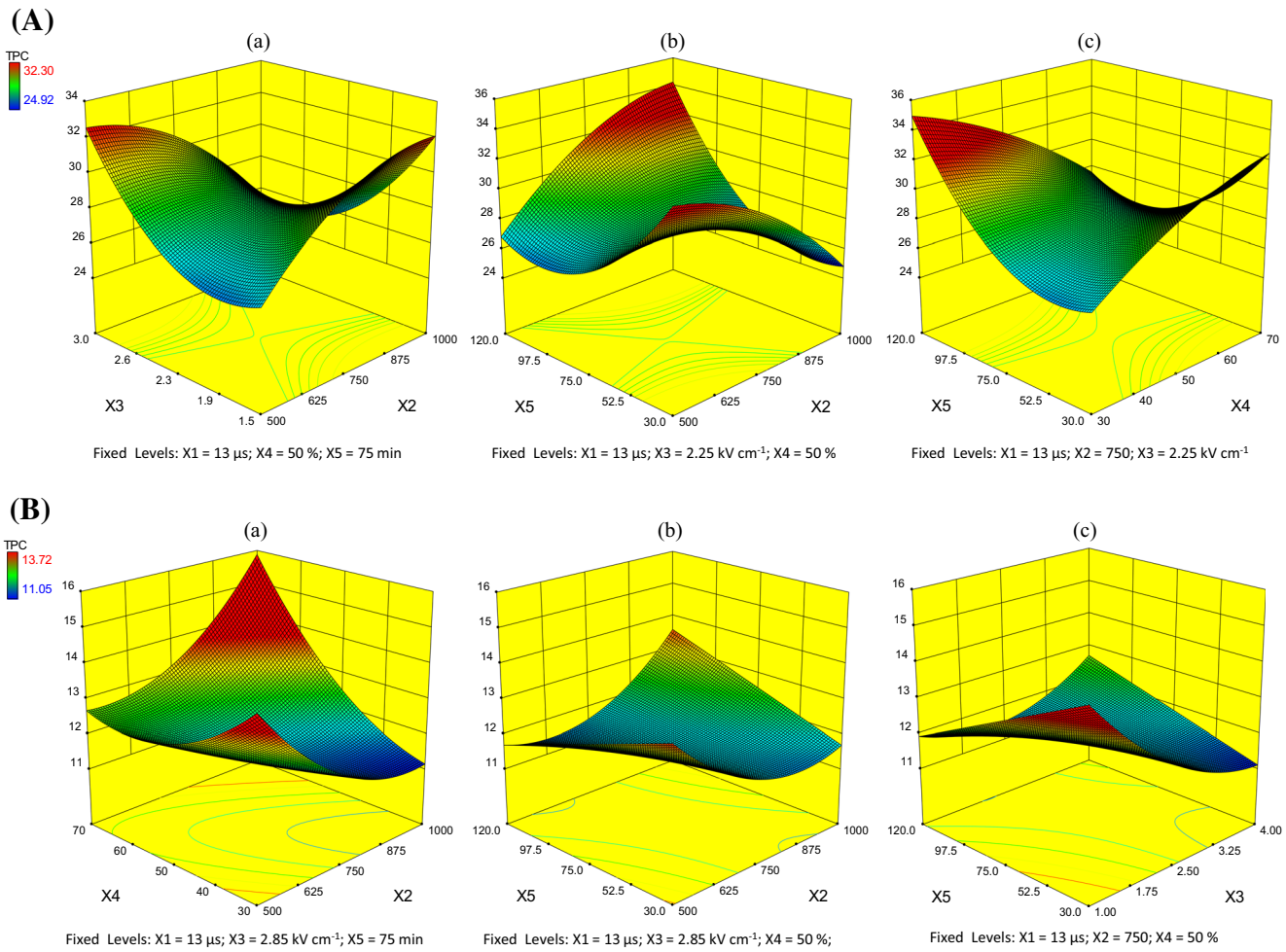

Fig. 1 Response surface and contour plots for the total phenolic compounds (TPC) yield with PEF-assisted extraction from CBS (**a**): (a) number of pulses vs PEP strength (X_2X_3); (b) number of pulses vs time of solid-liquid extraction (X_2X_5); (c) concentration of ethanol vs time of solid-liquid extraction (X_4X_5) and PEF-assisted extraction from CS (**b**): (a) number of pulses vs concentration of ethanol (X_2X_4); (b) number of pulses vs time of solid-liquid extraction (X_2X_5); (c) PEF strength vs time of solid-liquid extraction (X_3X_5)

temperatures, which can provide some turbidity to the extracts and compromise the purity of the extracts (Boussetta et al. 2009; Misra et al. 2017; Barba et al. 2015d). Thus, the selective recovery of polyphenols from CBS could be improved by PEF-assisted extraction without significant variations of temperature due to the very short processing time (microseconds) and the low energy input required for PEF permeabilisation.

Besides, high amounts of TPC can be yielded using again high number of pulses within long periods of solid-liquid extraction. The interaction of number of pulses with solid-liquid extraction time (X_2X_5) is shown in Fig. 1a (b). These results were in accordance with those found by Medina-Meza and Barbosa-Cánovas (2015) for the extraction of polyphenols from plum and grape, who observed that the use of high number of pulses significantly increased the extraction yield. The effectiveness of PEF to enhance the extraction process also depends on the extraction variables (time and concentration of the solvent), as well as the characteristics of the compound to be extracted (Puértolas and Barba 2016). As can be seen in Fig. 1a (c) (X_4X_5), although high extraction yields of polyphenols can be obtained with low periods of solid-liquid extraction time, which required high concentrations of ethanol, the highest yields were found using low concentrations of ethanol and longer extraction times, operating at moderate PEF strength for a short period of treatment (13 µs). In summary, highly significant results ($p < 0.01$) were achieved when using a high number of pulses combined with low PEF strength values, high number of pulses combined with long extraction times or long periods of extraction and low concentration of ethanol. Then, the PEF technology can be used to improve the extraction yield of phenolic compounds with low ethanol consumption, being more favourable from an environmental and economic point of view.

For the five evaluated variables, the optimum conditions for the extraction of phenolic compounds from CBS were as follows: PEF pre-treatment time of 11.99 µs, number of pulses of 991.28, PEF strength of 1.74 kV cm^{-1}, ethanol concentration of 39.15% and solid-liquid extraction time of 118.54 min.

The independent variables and maximum predicted values from the response surface curves correspond with the optimum values of the dependent variables obtained from the model equation. Under these conditions, the predicted TPC value for the CBS extract was 33.33 mg GAE g^{-1} dw, while the experimental values for TPC were 33.05 ± 3.13, showing that the model is satisfactory and accurate.

Model Fitting, Response Surface Analysis and Optimisation for CS

The total phenolic content (Y_{TPC}) in the CS extracts obtained from the 53 experiments is listed in Table 3 (b). The TPC values ranged from 11.05 (experiment run 27) and 13.72 mg GAE g^{-1} dw (experiment run 42). In this case, the variation in TPC for the CS matrix was less marked than for CBS and the increase in the extraction yields of polyphenols observed was up to 19% after PEF pre-treatment compared to control extraction. However, the PEF pre-treatment improved the extraction yields and reached similar TPC values as from solid-liquid extraction at 80 °C (12.81 mg GAE g^{-1} dw), as described by Ballesteros et al. (2014), or significantly higher TPC values than those found by Narita and Inouye (2012) (7.00 mg GAE g^{-1} dw) when operating at room temperature.

The significance of the coefficients of the model determined by analysis of variance (ANOVA) is shown in Table 4. For CS, the significant factors were the PEF strength ($p < 0.05$) and ethanol concentration ($p < 0.01$) as well as the quadratic effects of the PEF treatment time ($p < 0.05$), number of pulses, PEF strength and ethanol concentration ($p < 0.01$). For CS, the significant interactions were only the PEF treatment time/ethanol concentration ($p < 0.01$), number of pulses/ethanol concentration ($p < 0.05$), number of pulses/extraction time and PEF strength/extraction time ($p < 0.01$).

The model obtained by RSM that showed relationships between the TPC and extraction parameters for CS has a correlation coefficient of $R^2 = 0.7453$ and $R^2_{Adj.} = 0.6783$, which indicated a satisfactory correlation between the experimental values and those predicted by the equations. The obtained model is expressed by the following quadratic polynomial equations in the form of coded values:

$$\begin{aligned}\text{TPC (CS)} = {} & 20.83 - 2.596x_3 - 0.253x_4 - 0.056x_1x_4 \\ & + 0.014x_2x_4 - 0.031x_2x_5 + 0.092x_3x_5 \\ & + 0.015x_1^2 - 0.027x_2^2 + 0.299x_3^2 + 0.018x_4^2 \quad (5)\end{aligned}$$

Additionally, for CS, the statistical significance of the regression equation was evaluated by analysis of variance (ANOVA). The p value was less than 0.0001, which indicates that the model is significant and can be used to optimise the extraction of polyphenols for this by-product. The low value of the coefficient of variation (% CV = 3.02) indicated a high degree of precision and a good deal of reliability for the experimental values of the model for CS. Moreover, the lack of fit value was not significant ($p > 0.05$), which indicates that the fitting model is valid and adequate to describe the experimental data.

Three-dimensional response surfaces and two-dimensional contour plots were generated by the model for the results of the TPC in CS extracts to evaluate the most significant interactions of variables and their effect in the CCD (Fig. 1b). The plots were obtained depicting two variables within the experimental range, while the other three variables were fixed constant at their respective centre value of the testing ranges. Also for CS samples, the variable number of pulses was found highly significant ($p < 0.01$) to yield high amounts of phenolic compounds in combination with higher concentrations of ethanol (X_2X_4) (Fig. 1b (a)) than those used for CBS, and in less extent in combination with the solid-liquid extraction time (X_2X_5) ($p < 0.05$) (Fig. 1b (b)). Additionally, highly significant results ($p < 0.01$) were achieved when using low PEF strength values with a moderate extraction time (X_3X_5) (Fig. 1b (c)). As observed for CBS, the results supported the concept that in general, the electroporation effect does not require high power consumption and the use of the lowers PEF strength values can be adequate to obtain high extraction yields. However, in this case, to achieve the highest TPC yields, higher amounts of ethanol and moderate solid-liquid extraction time were required possibly due to the nature of the compounds extracted. For CS matrix, the RSM results highlighted that using high number of pulses in combination with moderate PEF treatment with lower values of PEF strength for a short period of treatment (microseconds), intermediate period of solid-liquid extraction time and higher concentration of ethanol result in extracts with high amounts of polyphenols. The initial temperature before PEF treatment was $T_i = 25$ °C and the final temperature after electrical treatment T_f never exceeded 27.5 °C ($\Delta T \leq 2.5$ °C) (see Table 3b). As explained before for CBS samples, also in this case, PEF-assisted extraction represents a green alternative to conventional extraction of bioactive compounds, reducing the possible losses of target molecules due to thermal degradation. Furthermore, the application of PEF treatment considerably enhances the diffusivity of valuable components in the vegetal tissues at room temperatures and may be an interesting alternative for the extraction of thermolabile molecules (Barba et al. 2015d).

For the five variables considered, the optimum conditions for the extraction of polyphenols from CS were as follows: PEF pre-treatment time of 5.45 μs, number of pulses of 1000, PEF strength of 1.37 kV cm^{-1}, ethanol concentration of 62.67% and solid-liquid extraction time of 75 min; the predicted TPC was 13.82 mg GAE g^{-1} dw. The experimental value for TPC was 12.12 ± 0.53 mg GAE g^{-1} dw, showing that the model is significantly more suitable for the CBS matrix than CS. As observed for CBS samples, also for CS

extraction procedure, the independent variables and maximum predicted values from the RSM correspond with the optimum values of the dependent variables obtained from the model equation.

Comparison of PEF-Assisted Extraction with Conventional Solid-Liquid Extraction

To evaluate the efficiency of the PEF pre-treatment, the optimised extraction procedure was applied to 12 samples of CBS and 12 samples of CS and then compared with the results of the conventional solvent extraction method without the PEF pre-treatment. All extracts were analysed for total phenolic compounds, flavonoids and tannins as well as the DPPH radical scavenging activity (Table 5). Furthermore, the main methylxanthines (theobromine and caffeine) and phenolic compounds (epicathechin and 5-caffeoylquinic acid) present in CBS and CS samples were identified and quantified by HPLC analysis, and the results are shown in Table 6.

The use of PEF pre-treatment improved the extraction of phenolics compared to conventional extraction in 75% of the CBS samples and 83% of the CS samples investigated.

The increases in the total phenolic contents (TPC) observed in extracts from several CBS and CS samples tested were 1.8–19.5 and 3–21%, respectively, compared with untreated samples. These results were in accordance with prior studies for other food by-products. Application of the PEF treatment in fermented grape pomace operating at a similar electric field intensity ($E = 1.2$ kV cm^{-1}) and higher extraction times increased the polyphenol extraction yield by 12.9% compared to untreated samples (Brianceau et al. 2015). Boussetta et al. (2014) described the application of PEF pre-treatment for the extraction of polyphenols from flaxseed hulls under several conditions and observed that the extraction yields of TPC increased between 12 and 38%. Under similar conditions as those used in the present study (ethanol 50% and identical time of extraction) and operating at a higher electric field intensity (20 kV cm^{-1}), these authors yielded extracts with total phenolic contents that were 12% higher than the control. Considering the specificity of each sample (origin, variety, industrial treatment and chemical composition), the enhancement of polyphenol extraction was generally higher for several CBS and CS samples than those described previously. Furthermore, PEF pre-treatment increased the extraction of flavonoids up to 20% in CBS samples and 21.3% in CS samples. The effect was more evident for CS samples; it was effective in more than 90% of the tested samples. This enhancement in extraction yields of flavonoids was higher than that observed in red raspberry puree by Medina-Meza et al. (2013). The PEF pre-treatment had a lower impact on the tannin content, except for in Java (JAV) and San Tome (STM) samples, which were higher than 10%. This group of compounds was not detected in the different extracts that yielded from CS samples. Data related to the PEF pre-treatment for the extraction of tannins are scarce in the literature. Application of PEF pre-treatment to improve the content of tannins (13.2%) in freshly fermented model wines was recently reported by El Darra et al. (2016). The use of PEF pre-treatment improved the antioxidant activity of extracts from 1.85% for CBS from Sao Tome up to 17% for CBS from Java. However, the results showed that PEF pre-treatment did not significantly affect the antioxidant activity ($p < 0.05$) of extracts obtained from CS samples compared to untreated samples, except for ADM, RDS and RWS, which increased by approximately 5%. PEF pre-treatment was more effective for the extraction of phenolic compounds from CBS samples from Venezuela, Java, Ecuador and Mexico. In CS samples PEF pre-treatment had significant effects on the yields of polyphenols from varietal Robusta (RDM and RWM) and Arabica dry hard roasting (ADH). Comparing the different matrices, CBS extracts had a higher level of active compounds (TPC = 17.88–55.16 mg g^{-1}, TTC = 8.08–25.30 mg g^{-1} and TFC = 6.44–43.94 mg g^{-1}) ($p < 0.05$) and higher antioxidant activity (RSA = 101.10–311.18 μM g^{-1}) than CS samples (TPC = 9.26–12.87 mg g^{-1}, TFC = 2.91–4.08 mg g^{-1}, TTC not detected, RSA = 45.65–71.3718 μM g^{-1}) ($p < 0.05$).

CBS extracts yielded from samples from Mexico, Honduras and Sao Tomé (MEX, HND and STM, respectively) displayed high levels of phenolic compounds (TPC 36.57–55.16 mg g^{-1}, tannins 18.47–25.30 mg g^{-1} and flavonoids 24.65–43.94 mg g^{-1}) and thus high antioxidant activity (RSA = 216.89–321.97 μM g^{-1}). Samples from Ecuador, Venezuela and Colombia (VNZ2, CLB1, ECD1 and ECD2) had lower levels of TPC and flavonoids. Data related to the level of phenolic compounds in cocoa bean shells from different origins are scarce in the literature. Bruna et al. (2009) reported, for the first time, the total content of polyphenols for cocoa bean hulls from five different origins. In the present study, knowledge of the polyphenol content in CBS was extended to more origins. Additionally, the TPC determined for samples from the same origin was significantly increased (up to ten times higher) after using PEF-assisted extraction compared with conventional solvent extraction (0.1% HCl/methanol) by previous authors. Moreover, the total phenolic content increased in the same order as in the present work: Ecuador < Venezuela < Madagascar < Trinidad, which confirmed that the differences observed in TPC could be related to the origin of cocoa. Flavonoids accounted for the main compounds present in CBS samples, which ranged from 50.3 to 79.7% of the total polyphenols according to the source of cocoa. The total phenolic compounds and flavonoid content were significantly correlated ($r = 0.9800$), while the correlations between TPC and tannins or flavonoids and tannins were $r = 0.7126$ and $r = 0.6752$, respectively. The radical scavenging activity of CBS samples was strongly correlated with the flavonoid content ($r = 0.9740$) and total phenolics ($r = 0.9647$)

Table 5 Antioxidant activity (RSA) and content of total phenolic compounds, flavonoids and tannins in extracts yielded from PEF-treated (PEF) and untreated (control) samples of cocoa bean shell (CBS) and coffee silverskin (CS)

Sample	Total phenolic content[a] (mg GAE g^{-1} dw)		Total flavonoids[a] (mg catechin g^{-1} dw)		Total tannins[a] (mg catechin g^{-1} dw)		RSA[a] (μM TE g^{-1} of dw)	
	PEF	Control	PEF	Control	PEF	Control	PEF	Control
CBS								
VNZ1	33.05 ± 3.13^{bA}	27.64 ± 2.13^{fB}	16.63 ± 0.94^{eA}	15.22 ± 1.66^{fB}	12.73 ± 0.51^{gA}	7.74 ± 0.70^{lB}	136.09 ± 4.47^{hA}	130.40 ± 9.65^{fB}
VNZ2	24.33 ± 1.04^{dA}	22.88 ± 0.97^{hB}	14.11 ± 0.86^{fA}	12.95 ± 0.82^{gB}	15.13 ± 0.46^{eA}	13.86 ± 0.39^{eB}	142.01 ± 5.73^{gA}	132.58 ± 2.52^{fB}
CLB1	22.71 ± 0.53^{eB}	25.48 ± 1.26^{gA}	13.14 ± 1.21^{fA}	13.46 ± 1.31^{gA}	7.24 ± 0.24^{lB}	8.47 ± 0.34^{iA}	118.24 ± 2.58^{iB}	129.27 ± 4.77^{fA}
CLB2	33.62 ± 0.60^{bA}	33.24 ± 1.32^{dA}	20.37 ± 1.85^{dA}	19.73 ± 1.33^{dA}	10.54 ± 0.40^{hA}	10.23 ± 0.43^{gA}	190.67 ± 1.42^{dA}	182.35 ± 2.88^{dB}
ECD1	21.13 ± 0.65^{fA}	19.83 ± 1.69^{iB}	13.28 ± 0.66^{fA}	11.56 ± 0.51^{hB}	14.51 ± 0.35^{fA}	13.79 ± 0.52^{eB}	117.52 ± 2.73^{iA}	109.06 ± 7.39^{gB}
ECD2	17.88 ± 0.87^{gA}	16.82 ± 0.51^{lB}	6.44 ± 0.49^{gA}	5.39 ± 0.35^{iB}	12.65 ± 0.26^{gA}	10.83 ± 0.19^{fB}	101.10 ± 2.80^{lA}	90.89 ± 1.61^{hB}
TND	33.74 ± 1.19^{bA}	30.28 ± 0.89^{eB}	21.76 ± 0.61^{cA}	19.42 ± 0.98^{dB}	8.08 ± 0.38^{iA}	8.26 ± 0.32^{iA}	176.95 ± 6.23^{eA}	162.76 ± 4.95^{eB}
JAV	33.10 ± 2.33^{bA}	29.40 ± 1.50^{efB}	20.39 ± 1.51^{dA}	17.30 ± 0.87^{eB}	10.45 ± 0.25^{hA}	9.16 ± 0.21^{hB}	188.20 ± 4.72^{dA}	159.76 ± 2.90^{eB}
MEX	54.08 ± 1.47^{aA}	47.62 ± 3.26^{bB}	39.11 ± 2.56^{bA}	37.14 ± 1.74^{bB}	21.03 ± 1.70^{bA}	20.10 ± 0.63^{bB}	321.97 ± 5.44^{aA}	285.71 ± 3.93^{bB}
HND	55.16 ± 2.24^{aA}	54.19 ± 2.57^{aA}	43.94 ± 2.83^{aA}	40.72 ± 1.62^{aB}	25.30 ± 0.71^{aA}	25.79 ± 0.89^{aA}	311.18 ± 9.79^{bB}	317.31 ± 6.74^{aA}
MGC	29.52 ± 1.30^{cA}	30.22 ± 0.98^{eA}	15.61 ± 0.75^{eB}	16.84 ± 1.02^{eA}	16.08 ± 0.46^{dB}	17.00 ± 0.64^{dA}	159.23 ± 15.35^{fB}	179.15 ± 4.9^{dA}
STM	33.01 ± 3.56^{bB}	36.57 ± 2.93^{cA}	22.46 ± 2.96^{cB}	24.65 ± 1.60^{cA}	18.47 ± 1.56^{cA}	18.02 ± 0.94^{cA}	216.89 ± 7.03^{cA}	212.97 ± 8.21^{cA}
CS								
ADS	12.03 ± 0.53^{bA}	11.20 ± 0.12^{aB}	3.19 ± 0.45^{defA}	3.12 ± 0.28^{cdA}	n.d.	n.d.	47.65 ± 2.28^{gB}	52.14 ± 2.50^{fA}
ADM	11.39 ± 0.77^{cA}	10.58 ± 0.48^{bcB}	3.13 ± 0.49^{efA}	2.58 ± 0.20^{fB}	n.d.	n.d.	66.14 ± 6.75^{bA}	42.36 ± 2.79^{gB}
ADH	11.19 ± 0.52^{cdA}	10.34 ± 0.38^{bcdB}	2.91 ± 0.46^{fA}	3.13 ± 0.47^{cdA}	n.d.	n.d.	62.98 ± 1.62^{bcdA}	63.88 ± 2.14^{cdA}
AWS	9.77 ± 0.39^{fA}	9.90 ± 0.63^{deA}	3.72 ± 0.70^{bcA}	3.53 ± 0.33^{bA}	n.d.	n.d.	64.31 ± 4.43^{bcB}	67.81 ± 5.03^{abA}
AWM	9.26 ± 0.44^{gA}	8.68 ± 0.31^{gB}	3.27 ± 0.39^{deA}	3.02 ± 0.36^{cdA}	n.d.	n.d.	62.67 ± 2.88^{cdA}	62.52 ± 1.72^{dA}
AWH	9.93 ± 0.83^{efA}	9.37 ± 0.67^{fB}	3.47 ± 0.23^{cdA}	3.25 ± 0.31^{cA}	n.d.	n.d.	63.64 ± 6.16^{bcdA}	63.41 ± 2.27^{dA}
RDS	10.27 ± 0.45^{eA}	9.50 ± 0.74^{efB}	3.32 ± 0.33^{deA}	2.78 ± 0.31^{efB}	n.d.	n.d.	54.59 ± 3.59^{fA}	51.68 ± 1.60^{fB}
RDM	12.02 ± 0.89^{bA}	10.80 ± 0.35^{abB}	3.98 ± 0.29^{abA}	3.80 ± 0.28^{aA}	n.d.	n.d.	57.63 ± 3.42^{eB}	60.08 ± 3.27^{eA}
RDH	11.57 ± 0.30^{bcA}	10.73 ± 0.81^{bB}	4.05 ± 0.27^{aA}	3.54 ± 0.27^{bB}	n.d.	n.d.	61.04 ± 1.98^{dA}	60.31 ± 3.48^{eA}
RWS	10.77 ± 0.49^{dA}	10.44 ± 1.00^{bcA}	3.19 ± 0.20^{defA}	2.76 ± 0.23^{efB}	n.d.	n.d.	63.42 ± 3.37^{bcdA}	60.10 ± 2.55^{eB}
RWM	12.87 ± 0.58^{aA}	10.60 ± 0.39^{bcB}	3.10 ± 0.42^{efA}	2.95 ± 0.21^{deA}	n.d.	n.d.	71.37 ± 2.47^{aA}	69.68 ± 4.25^{aA}
RWH	9.91 ± 0.37^{efA}	10.22 ± 0.52^{cdA}	3.24 ± 0.41^{deA}	2.75 ± 0.30^{efB}	n.d.	n.d.	64.97 ± 5.95^{bcA}	65.86 ± 4.15^{bcA}

TE Trolox equivalent, *GAE* gallic acid equivalent, *n.d.* not detected

[a] Mean values ($n = 3$) ± standard deviation followed by different lower case letters, superindexes within the same column and capital letters superindexes within the same row denote statistically significant differences at $p < 0.05$ (Duncan's test)

Table 6 Content (mg g^{-1} dw) of methylxanthines (theobromine and caffeine) and polyphenols (epicatechin and 5-caffeoylquinic acid) in cocoa bean shell (CBS) and coffee silverskin (CS) extracts yielded from PEF-treated (PEF) and untreated (control) samples of CBS and CS

Sample	Theobromine[a] (mg g^{-1} dw)		Caffeine[a] (mg g^{-1} dw)		Epicatechin[a] (mg g^{-1} dw)		5-Caffeoylquinic acid[a] (mg g^{-1} dw)	
	PEF	Control	PEF	Control	PEF	Control	PEF	Control
CBS								
VNZ1	7.38 ± 1.17^{cA}	8.04 ± 0.01^{cA}	4.21 ± 0.73^{aA}	4.08 ± 0.02^{aA}	0.74 ± 0.05^{deA}	0.59 ± 0.01^{hB}	n.a.	n.a.
VNZ2	6.28 ± 0.03^{dA}	5.59 ± 0.60^{gB}	2.03 ± 0.03^{efA}	1.71 ± 0.24^{dA}	0.31 ± 0.01^{hA}	0.21 ± 0.04^{gB}		
CLB1	4.64 ± 0.12^{eA}	4.67 ± 0.17^{hA}	1.63 ± 0.08^{fA}	1.95 ± 0.24^{dA}	0.45 ± 0.02^{ghA}	0.35 ± 0.01^{gB}		
CLB2	8.91 ± 0.22^{bA}	6.80 ± 0.34^{efB}	3.76 ± 0.34^{abA}	2.63 ± 0.25^{cB}	0.81 ± 0.05^{dA}	0.74 ± 0.08^{deA}		
ECD1	6.91 ± 0.60^{cdA}	6.70 ± 0.59^{fA}	1.70 ± 0.25^{fA}	1.71 ± 0.20^{dA}	1.00 ± 0.14^{cA}	0.90 ± 0.12^{cdA}		
ECD2	6.30 ± 0.16^{dA}	5.33 ± 0.60^{ghB}	1.59 ± 0.25^{fA}	1.61 ± 0.14^{dA}	0.49 ± 0.07^{fgA}	0.51 ± 0.03^{fA}		
TND	8.98 ± 0.32^{bA}	8.24 ± 0.67^{cA}	3.38 ± 0.59^{bcA}	3.32 ± 0.33^{bA}	0.60 ± 0.09^{efgA}	0.66 ± 0.03^{efA}		
JAV	7.27 ± 0.28^{cA}	6.77 ± 0.79^{efA}	2.54 ± 0.10^{deA}	1.99 ± 0.21^{dB}	1.00 ± 0.11^{cA}	0.99 ± 0.21^{cA}		
MEX	10.92 ± 0.33^{aA}	10.57 ± 0.54^{aA}	1.91 ± 0.18^{efA}	1.73 ± 0.24^{dA}	1.49 ± 0.13^{bA}	1.38 ± 0.15^{bA}		
HND	10.32 ± 0.15^{aA}	10.07 ± 0.13^{abA}	4.02 ± 0.14^{aA}	3.76 ± 0.14^{aA}	2.24 ± 0.08^{aA}	2.12 ± 0.05^{aA}		
MGC	6.76 ± 1.02^{cdA}	7.66 ± 0.06^{deA}	2.06 ± 0.50^{efA}	2.63 ± 0.01^{cA}	0.64 ± 0.02^{efA}	0.64 ± 0.01^{efA}		
STM	10.10 ± 0.16^{aA}	9.29 ± 0.82^{bA}	2.87 ± 0.02^{cdA}	2.78 ± 0.21^{cA}	0.74 ± 0.14^{deA}	0.89 ± 0.07^{cdA}		
CS								
ADS	n.a.	n.a.	4.44 ± 0.09^{fA}	4.31 ± 0.25^{hA}	n.a.	n.a.	0.48 ± 0.02^{deB}	0.54 ± 0.02^{dA}
ADM			4.59 ± 0.22^{fA}	4.32 ± 0.06^{hA}			0.33 ± 0.02^{gA}	0.31 ± 0.01^{fA}
ADH			4.60 ± 0.15^{fA}	4.45 ± 0.06^{ghA}			0.45 ± 0.02^{eA}	0.44 ± 0.02^{eA}
AWS			5.56 ± 0.28^{cdA}	5.71 ± 0.22^{cdeA}			1.03 ± 0.09^{aA}	1.11 ± 0.08^{aA}
AWM			5.14 ± 0.18^{eA}	5.23 ± 0.10^{fA}			0.79 ± 0.04^{bA}	0.78 ± 0.03^{bA}
AWH			5.04 ± 0.24^{eA}	4.72 ± 0.32^{gA}			0.75 ± 0.05^{bA}	0.69 ± 0.06^{cA}
RDS			5.77 ± 0.27^{bcA}	5.40 ± 0.13^{efA}			0.46 ± 0.05^{deA}	0.42 ± 0.02^{eA}
RDM			6.03 ± 0.16^{abA}	6.06 ± 0.16^{bcA}			0.53 ± 0.02^{dA}	0.54 ± 0.03^{dA}
RDH			6.07 ± 0.24^{abA}	5.84 ± 0.31^{bcdA}			0.60 ± 0.04^{cA}	0.59 ± 0.04^{dA}
RWS			6.23 ± 0.18^{aA}	6.13 ± 0.08^{bA}			0.41 ± 0.03^{efA}	0.41 ± 0.01^{eA}
RWM			5.23 ± 0.29^{deA}	5.59 ± 0.34^{defA}			0.36 ± 0.03^{fgA}	0.39 ± 0.05^{eA}
RWH			6.37 ± 0.17^{aA}	6.51 ± 0.32^{aA}			0.37 ± 0.01^{fgA}	0.39 ± 0.04^{eA}

n.a. not applicable

[a] Mean values ($n = 3$) ± standard deviation followed by different lower case letters superindexes within the same column and capital letters superindexes within the same row denote statistically significant differences at $p < 0.05$ (Duncan's test)

more than with tannins ($r = 0.729$). Other authors described similar correlations between active compounds and RSA for cocoa and cocoa by-products (Carrillo et al. 2014; Martínez et al. 2012). These results show that flavonoids could be related to the antioxidant activity of CBS extracts and, subsequently, that PEF pre-treatment could be used as a selective method for extracting flavonoids from this by-product. The effectiveness of PEF to enhance the extraction process depends on the PEF conditions, extraction variables, the by-product nature and the characteristics of the compound to be extracted. Several studies highlighted that PEF technology could be more effective on selective extraction of some groups of polyphenols such as flavonoids and anthocyanidins from foods and food by-products (Luengo et al. 2013; Medina-Meza and Barbosa-Cánovas 2015; Puértolas and Barba 2016). Additionally, the correlation between PEF-assisted extraction with antioxidant activity and different groups of polyphenols were already described in literature for grape peals (Medina-Meza and Barbosa-Cánovas 2015). These authors emphasised that PEF-assisted extraction, using moderate PEF treatment conditions similar to the present study, enhanced phenolics content with a consequent increase in antioxidant activity.

In general, CS extracts yielded from the Robusta variety using PEF pre-treatment displayed high levels of total phenolic compounds, while the Arabica variety had high levels of flavonoids. With respect to industrial processing, CS extracts obtained from Arabica dry-processed coffee had higher levels of TPC (11.19–12.03 mg g^{-1}) than samples derived from Arabica coffee treated with the wet method (9.26–9.93 mg g^{-1}); however, these last samples had higher levels of flavonoids (up to 3.72 mg g^{-1}) and a higher antioxidant activity (up to 64.31 μM g^{-1}). CS extracts derived from

Robusta coffee processed with dry or wet methods had similar TPC values (approximately 10.50 mg g^{-1}), while higher levels of flavonoids were found in CS extracts derived from Robusta coffee processed with the dry method (3.32–4.05 mg g^{-1}). Regarding the roasting process of coffee beans (soft, medium and hard), it was observed that the intensity of the roasting process reduced the TPC and TF values, which could be related to the low resistance of these compounds to temperature. However, in general, the antioxidant activity of the extracts increased. Hečimović et al. (2011) described similar variations in the total flavonoid and non-flavonoid contents in different coffees varieties (Arabica and Robusta) that were affected by different roasting processes (light, medium and dark). Moreover, these authors found that flavonoids represent approximately 44% of the total polyphenols present in roasted coffees. Similar portions of flavonoids were observed in the present study for CS samples (24.1–38.1%), which indicates that the largest portion of polyphenols was attributed to non-flavonoids. For CS samples, no correlation was observed between the RSA results and TPC or flavonoid contents ($r = -0.0633$ and -0.1661, respectively). This absence of a correlation was reported by other authors for coffee beverages prepared from beans roasted at different degrees and coffee silverskin (Alves et al. 2010; Costa et al. 2014). Alves et al. (2010) observed that the decrease in phenolic compounds in coffee beverages due to thermal degradation was not followed by the DPPH assay results, which were maintained or even increased, suggesting that the antioxidant activity could be due to the presence of other compounds than phenolics, such as melanoidins.

Epicatechin was the main phenolic compound present in CBS samples, as well as both methylxanthines theobromine and caffeine, while the main phenolic compound in CS samples was 5-caffeoylquinic acid in addition to methylxanthine caffeine.

PEF pre-treatment significantly improved the extraction yield of epicatechin for a low number of samples from Venezuela and Colombia (VNZ1, VNZ2 and CLB1) up to 30% higher than the control ($p < 0.05$).

These results were in accordance with other studies described in literature that confirm the potential of PEF-assisted extraction to increase the recovery of specific flavonoids such as epicatechin, resveratrol and kaempferol from wine shoots or catechin from grape skins (Rajha et al. 2014; Boussetta et al. 2009). The fact that PEF pre-treatment improved the recovery of epicatechin could be interesting as the biological effect depends on phenolic structure and it is necessary to evaluate the different phenolic compounds that are recovered from the different samples when any novel extraction technology is used and optimised.

The levels of epicatechin in CBS ranged from 0.31 to 2.24 mg g^{-1} according to geographic origin. This compound was quantified for CBS samples by HPLC for the first time in the present study. The epicatechin level determined in our study was higher for samples from Honduras (HND), Mexico (MEX) and Java (JAV), with values of 2.24, 1.49 and 1.00 mg g^{-1}, respectively. Bordiga et al. (2015) found similar values for cocoa samples from different geographical origins (ranging between 0.380 and 3.91). Considering Ecuador as the origin, these authors reported higher values of epicatechin for cocoa beans (4.51 mg g^{-1}) and comparable values for dark chocolate (0.578 mg g^{-1}) than those found for CBS from the same origin (1.00 and 0.49 mg g^{-1} for ECD1 and ECD2, respectively). Extracts yielded from Colombia CBS samples, CLB1 and CLB2, exhibited epicatechin contents of 0.45 and 0.81 mg g^{-1}, respectively, which represented 10–20% of the epicatechin content found in different cultivars of Colombian cocoa beans (1.405–3.562 mg g^{-1}) (Carrillo et al. 2014). The results confirmed that the content of epicatechin is correlated with the total phenolic content, flavonoid content and tannin content, with correlation coefficients of $r = 0.8449$, $r = 0.8852$ and $r = 0.7462$, respectively. The results also established the correlation between the epicatechin content and radical scavenging activity ($r = 0.8219$–0.8509) determined by the DPPH assay. This correlation was higher than that reported by Carrillo et al. (2014) between the ORAC assay and epicatechin contents ($r = 0.6770$) for cocoa beans. Therefore, the significant increase in antioxidant capacity found after the application of the PEF-assisted extraction in comparison to extraction control may be due to the presence of epicatechin.

5-Caffeoylquinic acid (5-CQA) is the main phenolic compound present in coffee and silverskin (Narita and Inouye 2012). The results showed that the content of this compound was not significantly improved by PEF-assisted extraction. The contents in 5-CQA were separately determined for different varieties and industrial treatments for the first time in the present study and the obtained values ranged from 0.33 to 1.03 mg g^{-1}. The highest values were found in samples derived from Arabica coffee processed with the wet method (up to 1.03 mg g^{-1} for a light roasting) and for Robusta coffee processed with the dry method (up to 0.60 mg g^{-1} for a hard roasting). These results were in accordance with those found by Narita and Inouye (2012) for a mix of CS for both varietals (Arabica and Robusta), who observed similar contents (1.00 mg g^{-1}) after an extraction procedure similar to the one employed in this study without PEF pre-treatment. Finally, a moderate correlation was observed between 5-caffeoylquinic acid and total flavonoids ($r = 0.4863$) as well as between 5-caffeoylquinic acid and the antioxidant activity displayed by extracts ($r = 0.4250$). These findings again supported that the antioxidant activity of CS extracts could be from compounds other than phenolic compounds.

The theobromine content was less significantly improved using PEF-assisted extraction, except for samples CLB2 from Colombia, VEN2 from Venezuela and ECD2 from Ecuador.

The level of theobromine in CBS samples changed with the origin of cocoa and varied between 4.64 ± 0.12 and 10.92 ± 0.33 mg g^{-1}dw ($p < 0.05$). Arlorio et al. (2005) found similar levels of this compound (12 g kg^{-1}) for a mix of cocoa bean hulls from different geographic regions (Ghana, Ecuador and Avorio Coast). However, no data are available in the literature for samples from a single origin. Samples from Colombia (CBL1), Venezuela (VNZ2) and Ecuador (ECD1 and ECD2) had low levels of theobromine, while those from Mexico (MEX), Honduras (HND) and Sao Tomé (STM) had high theobromine levels (see Table 6). The theobromine found for ECD1 and ECD2 from Ecuador (6.91 and 6.30 mg g^{-1}, respectively) was similar to that found by other authors for dark chocolate prepared with cocoa from the same origin (6.14 mg g^{-1}) (Bordiga et al. 2015). These authors described that the content of theobromine changed with the geographic origin and different processed samples of cocoa. Additionally, the amount of theobromine in CLB1 from Colombia (8.91 mg g^{-1}) agreed with that found by Carrillo et al. (2014) for cocoa samples from different cultivars (8.024–9.510 mg g^{-1}).

Caffeine was determined for both CS and CBS by-products. For this methylxanthine, PEF-assisted extraction had a slightly significant effect on the extraction yield. The level of caffeine CBS ranged between 1.59 and 4.21 mg g^{-1} and was generally similar to the levels found in cocoa by other authors. The caffeine level in CLB1 from Colombia agrees with that found by Carrillo et al. (2014) for cocoa samples from different cultivars (0.730–1.730 mg g^{-1}). Moreover, Bordiga et al. (2015) observed, for some samples from Ecuador, similar caffeine levels to those found in ECD2 (up to 1.37 mg g^{-1}). The results obtained confirm that the caffeine level depends on the origin of cocoa and different cultivars as well as that the content present in the CBS by-product is similar to that found in cocoa samples. Furthermore, we observed that the content of theobromine could be correlated with caffeine in CBS samples ($r = 0.500$) as well to the radical scavenging activity ($r = 0.8735$).

For CS samples, the caffeine level for varietal Robusta (raged from 5.23 to 6.35 mg g^{-1}) had higher caffeine levels than for varietal Arabica (from 4.44 to 5.56 mg g^{-1}). These results were similar to or higher than those found for CS mixed with both varietals extracted with acidic water at different temperatures (4.4 mg g^{-1}) by Narita and Inouye (2012). However, the caffeine levels for silverskin samples were lower than in coffee (8.2–25.2 mg g^{-1}) from different varietals, which were affected by different roasting degrees (Hečimović et al. 2011).

The composition of the different extracts on bioactive compounds (polyphenols and methylxanthines) and their antioxidant properties depend on the origin, variety and industrial processing of the raw material. The differences were highly significant for the CBS matrix (TPC of Honduras was 70% higher than Ecuador). Therefore, the collection of specific CBS monovarietal samples (e.g., MEX and HND from Mexico and Honduras, respectively) could be an interesting approach to achieve extracts with high phenolic compound levels and with high antioxidant activity.

Conclusions

This is the first study that provides information on the application of PEF technology for the extraction of high-added value compounds from cocoa and coffee by-products. The results highlighted the potential of PEF-assisted technology to improve the recovery of bioactive compounds from CBS and CS as a green extraction alternative to conventional extraction methods with feasible application at the industrial scale. The variables were significant, and the high correlation of the quadratic model obtained was satisfactory and accurately predicted the TPC for CBS and CS matrices. Properly selected parameters of PEF-assisted extraction specific for each matrix can enhance the extractability of bioactive compounds in CBS and CS samples up to 20 and 21.3%, respectively, compared with untreated samples and may be employed to produce extracts with high nutritional specific phytochemical profiles.

Furthermore, this study also highlighted that the contents of bioactive compounds in CBS and CS are highly correlated with different factors, such as the geographic origin, varietal or industrial treatment, as in the respective beans.

Although the levels of polyphenols and methylxanthines for CBS and CS were generally lower than in raw materials (cocoa and coffee beans), the important yield levels justifies the revalorisation of these by-products as new sources for the recovery of these compounds with beneficial effects and with several applications in the food (as food ingredients and nutraceuticals), cosmetic and pharmaceutical industries.

Acknowledgements This project received funding from the European Union's Seventh Framework programme for research and innovation under the Marie Skłodowska-Curie grant agreement No 609402 - 2020 researchers: Train to Move (T2M).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

References

Alves, R. C., Costa, A. S., Jerez, M., Casal, S., Sineiro, J., Núñez, M. J., & Oliveira, B. (2010). Antiradical activity, phenolics profile, and hydroxymethylfurfural in espresso coffee: influence of technological factors. *Journal of Agricultural and Food Chemistry, 58*(23), 12221–12229. https://doi.org/10.1021/jf1031229.

Anderson, K. A., & Smith, B. W. (2002). Chemical profiling to differentiate geographic growing origins of coffee. *Journal of Agricultural*

and Food Chemistry, 50(7), 2068–2075. https://doi.org/10.1021/jf011056v.

Arlorio, M., Coïsson, J. D., Travaglia, F., Varsaldi, F., Miglio, G., Lombardi, G., & Martelli, A. (2005). Antioxidant and biological activity of phenolic pigments from Theobroma cacao hulls extracted with supercritical CO 2. *Food Research International, 38*(8-9), 1009–1014. https://doi.org/10.1016/j.foodres.2005.03.012.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurulb, M. H. A., Ghafoorc, K., Norulainid, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: a review. *Journal of Food Engineering, 117*(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014.

Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. (2014). Selection of the solvent and extraction conditions for maximum recovery of antioxidant phenolic compounds from coffee silverskin. Food and bioprocess technology, 7(5), 1322–1332. https://doi.org/10.1007/s11947-013-1115-7

Barba, F. J., Brianceau, S., Turk, M., Boussetta, N., & Vorobiev, E. (2015a). Effect of alternative physical treatments (ultrasounds, pulsed electric fields, and high-voltage electrical discharges) on selective recovery of bio-compounds from fermented grape pomace. *Food and Bioprocess Technology, 8*(5), 1139–1148. https://doi.org/10.1007/s11947-015-1482-3.

Barba, F. J., Galanakis, C. M., Esteve, M. J., Frigola, A., & Vorobiev, E. (2015b). Potential use of pulsed electric technologies and ultrasounds to improve the recovery of high-added value compounds from blackberries. *Journal of Food Engineering, 167*, 38–44. https://doi.org/10.1016/j.jfoodeng.2015.02.001.

Barba, F. J., Grimi, N., & Vorobiev, E. (2015c). Evaluating the potential of cell disruption technologies for green selective extraction of antioxidant compounds from Stevia rebaudiana Bertoni leaves. *Journal of Food Engineering, 149*, 222–228. https://doi.org/10.1016/j.jfoodeng.2014.10.028.

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J. A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N., & Vorobiev, E. (2015d). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International, 77*, 773–798. https://doi.org/10.1016/j.foodres.2015.09.015.

Bobinaitė, R., Pataro, G., Lamanauskas, N., Šatkauskas, S., Viškelis, P., & Ferrari, G. (2015). Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. *Journal of Food Science and Technology, 52*(9), 5898–5905. https://doi.org/10.1007/s13197-014-1668-0.

Bordiga, M., Locatelli, M., Travaglia, F., Coïsson, J. D., Mazza, G., & Arlorio, M. (2015). Evaluation of the effect of processing on cocoa polyphenols: Antiradical activity, anthocyanins and procyanidins profiling from raw beans to chocolate. *International Journal of Food Science & Technology, 50*(3), 840–848. https://doi.org/10.1111/ijfs.12760.

Boussetta, N., Lebovka, N., Vorobiev, E., Adenier, H., Bedel-Cloutour, C., & Lanoisellé, J. L. (2009). Electrically assisted extraction of soluble matter from chardonnay grape skins for polyphenol recovery. *Journal of Agricultural and Food Chemistry, 57*(4), 1491–1497. https://doi.org/10.1021/jf802579x.

Boussetta, N., Soichi, E., Lanoisellé, J. L., & Vorobiev, E. (2014). Valorization of oilseed residues: extraction of polyphenols from flaxseed hulls by pulsed electric fields. *Industrial Crops and Products, 52*, 347–353. https://doi.org/10.1016/j.indcrop.2013.10.048.

Brianceau, S., Turk, M., Vitrac, X., & Vorobiev, E. (2015). Combined densification and pulsed electric field treatment for selective polyphenols recovery from fermented grape pomace. *Innovative Food Science & Emerging Technologies, 29*, 2–8. https://doi.org/10.1016/j.ifset.2014.07.010.

Bruna, C., Eichholz, I., Rohn, S., Kroh, L. W., & Huyskens-Keil, S. (2009). Bioactive compounds and antioxidant activity of cocoa hulls (Theobroma cacao L.) from different origins. *Journal of Applied Botany and Food Quality, 83*, 9–13.

Butt, M. S., & Sultan, M. T. (2011). Coffee and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition, 51*(4), 363–373. https://doi.org/10.1080/10408390903586412.

Carrillo, L. C., Londoño-Londoño, J., & Gil, A. (2014). Comparison of polyphenol, methylxanthines and antioxidant activity in Theobroma cacao beans from different cocoa-growing areas in Colombia. *Food Research International, 60*, 273–280. https://doi.org/10.1016/j.foodres.2013.06.019.

Costa, A. S., Alves, R. C., Vinha, A. F., Barreira, S. V., Nunes, M. A., Cunha, L. M., & Oliveira, M. B. P. (2014). Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. Industrial Crops and Products, 53, 350–357. https://doi.org/10.1016/j.indcrop.2014.01.006

Donsì, F., Ferrari, G., & Pataro, G. (2010). Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *Food Engineering Reviews, 2*(2), 109–130. https://doi.org/10.1007/s12393-010-9015-3.

Dorenkott, M. R., Griffin, L. E., Goodrich, K. M., Thompson-Witrick, K. A., Fundaro, G., Ye, L., Stevens, J. R., Mostafa, A., O'Keefe, S. F., Hulver, M. W., & Neilson, A. P. (2014). Oligomeric cocoa procyanidins possess enhanced bioactivity compared to monomeric and polymeric cocoa procyanidins for preventing the development of obesity, insulin resistance, and impaired glucose tolerance during high-fat feeding. *Journal of Agricultural and Food Chemistry, 62*(10), 2216–2227. https://doi.org/10.1021/jf500333y.

El Darra, N., Turk, M. F., Ducasse, M. A., Grimi, N., Maroun, R. G., Louka, N., & Vorobiev, E. (2016). Changes in polyphenol profiles and color composition of freshly fermented model wine due to pulsed electric field, enzymes and thermovinification pretreatments. *Food Chemistry, 194*, 944–950. https://doi.org/10.1016/j.foodchem.2015.08.059.

Guglielmetti, A., D'Ignoti, V., Ghirardello, D., Belviso, S., & Zeppa, G. (2017). Optimisation of ultrasound and microwave-assisted extraction of caffeoylquinic acids and caffeine from coffee silverskin using response surface methodology. *Italian Journal of Food Science, 29*, 409–423. https://doi.org/10.14674/IJFS-727.

Hečimović, I., Belščak-Cvitanović, A., Horžić, D., & Komes, D. (2011). Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. *Food Chemistry, 129*(3), 991–1000. https://doi.org/10.1016/j.foodchem.2011.05.059.

Herald, T. J., Gadgil, P., & Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture, 92*(11), 2326–2331. https://doi.org/10.1002/jsfa.5633.

Herald, T. J., Gadgil, P., Perumal, R., Bean, S. R., & Wilson, J. D. (2014). High-throughput microplate HCl–vanillin assay for screening tannin content in sorghum grain. *Journal of the Science of Food and Agriculture, 94*(10), 2133–2136. https://doi.org/10.1002/jsfa.6538.

Kaplinsky, R. (2004). *Competitions policy and the global coffee and cocoa value chains*. Geneva: UNCTAD.

Luengo, E., Álvarez, I., & Raso, J. (2013). Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innovative Food Science & Emerging Technologies, 17*, 79–84. https://doi.org/10.1016/j.ifset.2012.10.005.

Martín, M. A., & Ramos, S. (2016). Cocoa polyphenols in oxidative stress: potential health implications. *Journal of Functional Foods, 27*, 570–588. https://doi.org/10.1016/j.jff.2016.10.008.

Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2012). Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products. *Food Research International, 49*(1), 39–45. https://doi.org/10.1016/j.foodres.2012.08.005.

Martínez-Pinilla, E., Oñatibia-Astibia, A., & Franco, R. (2015). The relevance of theobromine for the beneficial effects of cocoa consumption. *Frontiers in Pharmacology, 6*, 1–5. https://doi.org/10.3389/fphar.2015.00030.

Medina-Meza, I. G., & Barbosa-Cánovas, G. V. (2015). Assisted extraction of bioactive compounds from plum and grape peels by ultrasonics and pulsed electric fields. *Journal of Food Engineering, 166*, 268–275. https://doi.org/10.1016/j.jfoodeng.2015.06.012.

Medina-Meza, I. G., Boioli, P., & Barbosa-Cánovas, G. V. (2013). Assessment of the effects of ultrasonics and pulsed electric fields on nutritional and rheological properties of raspberry and blueberry purees. *Food and Bioprocess Technology, 9*(3), 520–531. https://doi.org/10.1007/s11947-015-1642-5.

Misra, N. N., Martynenko, A., Chemat, F., Paniwnyk, L., Barba, F. J., & Jambrak, A. R. (2017). Thermodynamics, transport phenomena and electrochemistry of external field assisted non-thermal food technologies. *Critical Reviews in Food Science and Nutrition*, 1–32. https://doi.org/10.1080/10408398.2017.1287660.

Murthy, P. S., & Naidu, M. M. (2012). Sustainable management of coffee industry by-products and value addition—a review. *Resources, Conservation and Recycling, 66*, 45–58. https://doi.org/10.1016/j.resconrec.2012.06.005.

Mussatto, S. I., Machado, E. M., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology, 4*(5), 661–672. https://doi.org/10.1007/s11947-011-0565-z.

Narita, Y., & Inouye, K. (2012). High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. *Food Chemistry, 135*(3), 943–949. https://doi.org/10.1016/j.foodchem.2012.05.078.

Parniakov, O., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2014). Impact of pulsed electric fields and high voltage electrical discharges on extraction of high-added value compounds from papaya peels. *Food Research International, 65*, 337–343. https://doi.org/10.1016/j.foodres.2014.09.015.

Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev, E. (2015a). Pulsed electric field and pH assisted selective extraction of intracellular components from microalgae Nannochloropsis. *Algal Research, 8*, 128–134. https://doi.org/10.1016/j.algal.2015.01.014.

Parniakov, O., Roselló-Soto, E., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2015b). New approaches for the effective valorization of papaya seeds: extraction of proteins, phenolic compounds, carbohydrates, and isothiocyanates assisted by pulsed electric energy. *Food Research International, 77*, 711–717. https://doi.org/10.1016/j.foodres.2015.03.031.

Parniakov, O., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2016). Extraction assisted by pulsed electric energy as a potential tool for green and sustainable recovery of nutritionally valuable compounds from mango peels. *Food Chemistry, 192*, 842–848. https://doi.org/10.1016/j.foodchem.2015.07.096.

Patras, M. A., Milev, B. P., Vrancken, G., & Kuhnert, N. (2014). Identification of novel cocoa flavonoids from raw fermented cocoa beans by HPLC–MS^n. *Food Research International, 63*, 353–359. https://doi.org/10.1016/j.foodres.2014.05.031.

Puértolas, E., & Barba, F. J. (2016). Electrotechnologies applied to valorization of by-products from food industry: main findings, energy and economic cost of their industrialization. *Food and Bioproducts Processing, 100*, 172–184. https://doi.org/10.1016/j.fbp.2016.06.020.

Quagliariello, V., Iaffaioli, R. V., Falcone, M., Ferrari, G., Pataro, G., & Donsì, F. (2016). Effect of pulsed electric fields-assisted extraction on anti-inflammatory and cytotoxic activity of brown rice bioactive compounds. *Food Research International, 87*, 115–124. https://doi.org/10.1016/j.foodres.2016.07.005.

Rajha, H. N., Boussetta, N., Louka, N., Maroun, R. G., & Vorobiev, E. (2014). A comparative study of physical pretreatments for the extraction of polyphenols and proteins from vine shoots. *Food Research International, 65*, 462–468. https://doi.org/10.1016/j.foodres.2014.04.024.

Sarkis, J. R., Boussetta, N., Blouet, C., Tessaro, I. C., Marczak, L. D. F., & Vorobiev, E. (2015). Effect of pulsed electric fields and high voltage electrical discharges on polyphenol and protein extraction from sesame cake. *Innovative Food Science & Emerging Technologies, 29*, 170–177. https://doi.org/10.1016/j.ifset.2015.02.011.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Steinberg, F. M., Bearden, M. M., & Keen, C. L. (2003). Cocoa and chocolate flavonoids: implications for cardiovascular health. *Journal of the American Dietetic Association, 103*(2), 215–223. https://doi.org/10.1053/jada.2003.50028.

Upadhyay, R., Ramalakshmi, K., & Rao, L. J. M. (2012). Microwave-assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry, 130*(1), 184–188. https://doi.org/10.1016/j.foodchem.2011.06.057.

von Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (Aspalathus linearis), a-tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry, 45*(3), 632–638. https://doi.org/10.1021/jf960281n.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology, 17*(6), 300–312. https://doi.org/10.1016/j.tifs.2005.12.004.

Wiktor, A., Sledz, M., Nowacka, M., Rybak, K., Chudoba, T., Lojkowski, W., & Witrowa-Rajchert, D. (2015). The impact of pulsed electric field treatment on selected bioactive compound content and color of plant tissue. *Innovative Food Science & Emerging Technologies, 30*, 69–78. https://doi.org/10.1016/j.ifset.2015.04.004.