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ARTICLE

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Two extraction approaches were compared, ultrasound-assisted extraction (UAE) and conventional extraction (CE), for the recovery of natural antioxidant phenolic compounds from spent coffee grounds (SCG), which could be used as cosmetics additives or nutritional supplements. Two factorial designs were used to investigate the effect of the varied process parameters: ultrasound power (50-400 W), ethanol content in the solvent (0-50 vol%) and solid to liquid ratio (20-40 mL / g.dm) for UAE, temperature (20-70 °C) and ethanol content in the solvent (0-50 vol%) for CE. Performances of UAE and CE processes were evaluated and compared regarding total polyphenols recovery, antioxidant activity of the obtained extracts and energy consumption of the process. Polyphenols recovery efficiency was influenced mostly by ethanol content in the solvent, then ultrasound power in the case of UAE and heating temperature in the case of CE. Optimal operating conditions were identified as 400W ultrasound power, 50 vol% ethanol in the solvent and 40 mL / g.dm liquid to solid for UAE, and about 50 °C heating temperature and 50 vol% ethanol content in the solvent for CE. Under these optimal conditions for each process, more than 83% and 64% of available polyphenols in SCG were recovered with UAE and CE, respectively. Ultrasound assistance thus allowed about 33% enhancement of polyphenols recovery, while dividing by more than 2 the energy consumption. Hence, ultrasound assisted extraction process was demonstrated to be an efficient and sustainable method to recover antioxidant polyphenols from spent coffee grounds.

1. Introduction

The valorization of by-products from the agri-food sector represents a major challenge of our century. Circular economy is one of the ways to limit the impact of human consumption on the environment and thus reduce food and bioresources waste. Food Waste Recovery is a key part of the circular economy and aims to deplete a by-product, also defined as a biowaste (often treated as waste), using processes with a low energy impact.[1,2] With around 7 million tons generated per year, spent coffee grounds represent an important source of by-products. Indeed, coffee is widely consumed throughout the world, with an annual consumption estimated at about 10 millions of tons by the International Coffee.[3] This biowaste is undervalued, since its applications are limited to direct use as compost, as substrate for the production of edible mushrooms, or as pellets.[4–6] Many by-products, like SCG, are still rich in high value molecules including polyphenols. These biomolecules exhibit antioxidant activities enabling a variety of applications in cosmetics, medicine, pharmacology or agri-food fields.[7] The main polyphenols reported to be present in spent coffee grounds are chlorogenic acid and its derivatives such as caffeoylquinic acid, p-coumaroylquinic acid or feruloylquinic acid.[8,9] In pharmacology, these compounds have been studied for their potential activity against human chronic degenerative diseases, cancer and cardiovascular disease.[10] They can also be used as a chemopreventive agent to treat neurodegenerative disorders.[11] In food industry, natural polyphenols are used to replace synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ), which have been reported to cause health risks.[12] To propose sustainable valorization of by-products, extraction of high added value molecules is studied using innovative processes, such as ultrasound-assisted extraction. These technologies are commonly considered as green processes since good extraction yields were obtained with less residence time and energy consumption. They also permit the reduction of solvent quantities and favour the use of GRAS solvents.[1,13–18]

The aim of this work was to propose a sustainable valorization of polyphenols from SCG, using ultrasound-assisted extraction (UAE), as it is recognized as an efficient eco-process in several

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similar applications.[19,20] In previous studies published on UAE of polyphenols from SCG[21,22], UAE performances were generally compared to Soxhlet, maceration or even conventional solid-liquid extractions (with heated solvent), but not considered in optimized operating conditions. In the present study, UAE was compared to a conventional extraction (CE), so as to understand the impact of US assistance on extraction performances. Hence, CE were performed with heated solvent at similar temperatures than those observed with US application. Optimization of UAE and CE were carried out using experimental designs methodology. Many factors can influence bioactive compounds' recovery efficiency in solid-liquid extraction: type of solvent, liquid to solid ratio, temperature, ultrasound power of UAE, extraction time, particle size.[1,23–25] Experimental design was used to identify the most influential factors and optimal operating conditions, while limiting experimental runs.[26] Preliminary studies were carried out to characterize SCG solid source and to define relevant ranges of variation for some parameters, before performing designs of experiments. Optimization and comparison of UAE and CE were conducted in terms of polyphenols recovery, antioxidant activity of the obtained extracts, energy consumption and solvent temperature.

2. Materials and methods

2.1 Chemicals

Ethanol was purchased from VWR (Fontenay-sous-Bois, France). Sodium carbonate was purchased from Prolabo (Paris, France). The Folin-Ciocalteu's phenol reagent was obtained from EMD Milipore Corporation (Billerica, Ma, USA). DPPH (2,2 Diphenyl-1-picrylhdrazyl), Trolox (6-hydroxy 2,5,7,8 tetramethylchroman-2-carboxylic acid), gallic acid (3,4,5-trihydroxybenzoic acid) and potassium persulfate were obtained from Sigma Aldrich Co. (St. Louis, Mo, USA). ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was purchased from Alfa Aesar (Ward Hill, MA, USA). All chemicals were analytical grade, with purities greater than 99%, except for ABTS (98%). Aqueous solutions were prepared with distilled water.

2.2. Spent coffee grounds

Spent coffee grounds were provided by a local waste collection company, Gecco (Avelin, France). This company collects a mix of spent coffee grounds (SCG) from restaurants and bars in the North of France. The batch of SCG used for the present work had been stored on Gecco site in open air barrels for approximately 1-6 months. To prevent microbial spoilage during storage at laboratory, SCG were dried in an electric forced-air food dehydrator (Food Dehydrator, Excalibur) at 40 °C for 12 hours. Dried SCG were then placed in glass containers and stored in the dark at room temperature until use.

2.3. Extraction procedure

Equipment used to carry out conventional extractions (CE) and ultrasound-assisted extractions (UAE) were schematically presented in Figure 1.

All extractions were carried out in batch mode. For conventional extraction (CE), a 1 L Pyrex flat-bottomed glass jacketed reactor was used, connected to a thermostatically controlled bath to regulate temperature (Fisherbrand, Isotemp 5150 H7). A mechanical stirrer (IKA, EUROSTAR 60) and a 4-blade impeller (IKA, R1342) were used for stirring. For UAE, a 0.9 L stainless steel pipe with a 20 kHz frequency generator (SINAPTEC, Ultrasonic Lab750) was used. A mechanical stirrer (IKA, RW 20 DZM) and a 3-blade impeller (IKA, R1382) were used for agitation. All UAE assays started at ambient temperature. Ultrasound assistance provoked a progressive increase in temperature. To prevent evaporation and heating issues, an operating limit was set: when 70 °C were reached, ultrasounds were stopped until temperature dropped below 68 °C, and then were restarted (US in discontinuous mode between 68 and 70 °C). All extractions (CE and UAE) were performed for a duration of 90 min with a stirring at 160 rpm (allowed a sufficient suspension and homogenisation of the solid in the liquid) in solvent volume fixed at 700 mL. 2 mL samples were collected every 5 minutes for the first 30 minutes and then every 30 minutes until the end of the 90 min. The samples were centrifugated for 60 seconds at 8500 g, filtered on a 0.45 µm sterile cellulose nitrate filter and stored at 4 °C until analysis. All samples were collected in duplicate for each experiment. For each sampling time, electricity consumption was measured with a power consumption controller (Otio, CC 5000) to which all electrical appliances used for extraction were connected. The temperature was measured with a thermocouple (TCDIRECT, Type K) and a portable data logger (GRAPHTEC, GL800).
Preliminary tests (section 3.1.2) were performed in 100 mL narrow-necked Erlenmeyer flasks with a working volume of 50 mL. The agitation was set at 150 rpm and maintained with an incubator (INFORST HT, Multitron strandard) for 20 hours at room temperature (RT).

2.4. Analytical measurements

2.4.1. Characterization of SCG

Moisture content of SCG was measured by a moisture analyzer (PRECISA, XM60). Evaluation of the initial phenolic content of SCG was performed according to the procedure described by Zuorro and Lavecchia[27] with some modifications. Briefly, a four-stage extraction procedure was performed.

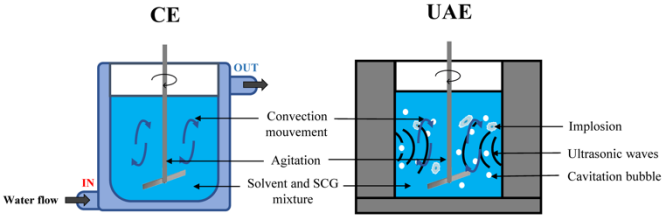

Figure 1. Schematic representation of equipment used for conventional extractions (CE) and ultrasound-assisted extractions (UAE) experiments.

The extraction was conducted at 70 °C in 200 mL magnetically stirred flasks containing 1 g of dry material (DM) and different volumes of 50% (v/v) ethanol in water solvent: 100, 50, 35 and 30 mL were used in the first, second, third and fourth stage, respectively. The contact time was set to 1 h in each stage. At the end of each extraction, the suspension was centrifugated for 10 min at 4800 g and filtered on a 0.45 µm cellulose nitrate filter. The solid phase was recovered for re-extraction and the liquid phase was stored at 4 °C until phenolic content and antioxidant activity assays, which were performed according to procedures described in section 2.4.2. The total amount of phenolics and antioxidant activity were determined as the sum of values obtained in the four stages.

2.4.2. Total Polyphenols Content (TPC) and Antioxidant Activity (AA) characterization of extracts

TPC and AA characterization of the extracts were performed according to Folin-Ciocalteu and DPPH methods, respectively, using high-throughput microplate procedures. Assays were carried out on a microplate reader (Thermo Fisher Scientific, Multiskan spectrum) using spectrophotometric detection and flat bottom 96-well microtiter plates.[28]

Folin-Ciocalteu microplate assays were performed according to the procedure described by Boizot and Charpentier[29] with some modifications. Briefly, a 20% (w/v) sodium carbonate solution was prepared. Folin-Ciocalteu's phenol reagent was diluted with water at a ratio of 1:5 (v/v). Aqueous standard solutions of gallic acid (50-400 mg / L) were daily prepared by dilution from a 400 mg / L stock solution. A calibration curve relating to this concentration range of gallic acid was established for each plate. 20 µL of extraction sample or gallic acid standard solution, 120 µL of water and 30 µL of Folin-Ciocalteu reagent solution (1:5, v/v) were placed in each well and mixed. After that, 30 µL of 20% (w/v) sodium carbonate solution was added. All experiments were performed in triplicate at 45 ± 1 °C. The absorbance at 765 nm of the blue complex formed was read after 8 min which was determined as the optimal time to get the maximal absorbance from the extracts. The reagent blank was set by the addition of 20 µL of water instead of standard solution or sample. The total phenolic content was expressed in gallic acid equivalent (mg of GAE / g dry matter) using the gallic acid calibration curve.

DPPH microplate assays were performed according to the procedure described by Ng and Owusu-Apenten[30] with some modifications. Briefly, a daily solution of DPPH at 0.04 g / L (optical density about 0.8 ± 0.05 at 515 nm) was prepared in ethanol and placed in the dark until use. Standard solutions of Trolox (125–1000 µM) were daily prepared by dilution with ethanol from a 1000 µM stock solution. A calibration curve relating this concentration range of Trolox was established for each plate. 10 µL of extraction sample or Trolox standard solution and 190 µL of DPPH solution were added in each well and mixed. All experiments were performed in triplicate at room temperature (25 ± 1 °C). The absorbance at 515 nm of the purple complex formed was read after 6 minutes. The reagent blank was set by the addition of 10 µL of ethanol instead of standard solution or sample. The total antioxidant activity was expressed in Trolox equivalent antioxidant capacity (TEAC) (µmol of Trolox / g dry matter) using the Trolox calibration curve.

2.5. Experimental designs

Two different face-centered central composite designs were used, the first for UAE and the second for CE. These two experimental designs include a full factorial design 2^k, where 2^k experiments were required to cover all possible combinations of factor levels.[31,32] For UAE experimental design, 3 factors (k = 3) and 2 levels were defined. Process variables were the ultrasound power (P, W), the liquid to solid ratio (R, mL of solvent / g of DM SCG) and the ethanol content in the solvent (E, vol%). For CE experimental design, 2 factors (k = 2) and 2 levels were defined. Process variables were the temperature (T, °C) and the ethanol content in the solvent (E, vol%). Liquid to solid ratio was fixed at 40 mL / g.dm. Real and coded values of low and high levels for each process variable were given in Table 1 for the UAE design, and in Table 2 for the CE design. Axial points (α) for both experimental designs were fixed to 1. Experimental errors were estimated thanks to central point repetition: 3 times for UAE design and 2 times for CE design. The 17 assays of the UAE design and the 10 assays of the CE design are fully detailed in Tables 3 and 4, respectively (in section 3.2.2).

For both UAE and CE experimental designs, process responses were total polyphenol content in the extract (TPC, mg GAE / g.dm), antioxidant activity of the extract (AA, µmol Trolox / g.dm), energy consumption (EC, W.h) and solvent temperature (ST, °C). Modelling and statistical analysis were performed with Design-expert V13 software (Stat-Ease, Minneapolis, USA). Each response was modelled using multiple regression according to a second-order polynomial function:

$$Y = b_0 + \Sigma_i b_i X_i + \Sigma_i b_{ii} X_i^2 + \Sigma_{ij} b_{ij} X_i X_j + \varepsilon$$

Y is the matrix of the answers, b_0 the constant value, b_i the coefficient effect of the factor X_i, b_{ij} represents the coefficient of interaction between factor X_i and factor X_j, and ε is the experimental error. Coefficients b_0, b_i, b_{ii} and b_{ij} are determined by matrix algebra according to the relation:

$$b = (X^t.X)^{-1}X^t.Y$$

X is the experiment matrix in coded variables and X^t is the transposed experiment matrix.

Analysis of variance (ANOVA) was used to evaluate the statistical significance of independent variables. If p-Value was less than 0.05 then the variables were considered significant.

Table 1. Level values for each process parameter of the UAE experimental design

Real values			Coded values		
P Watt	R mL / g.dm	E vol%	X_1	X_2	X_3
50	20	0	-1	-1	-1
225	30	25	0	0	0
400	40	50	+1	+1	+1

Table 2. Level values for each process parameter of the CE experimental design

Real values		Coded values	
T °C	E vol%	X_1	X_2
20	0	-1	-1
45	25	0	0
70	50	+1	+1

Using multilinear regression analysis (MLR), it was possible to perform a multivariate data analysis. Quadratic regression models were chosen so as to keep an adjusted R^2 value of at least 0.9.

3. Result & discussion

In order to propose a sustainable valorization of polyphenols from SCG, UAE was studied and compared with CE performed with heated solvent at similar temperatures than those observed with UAE, namely up to about 70 °C at maximal US power of 400W for the equipment used in this work.
Preliminary studies were carried out to characterize SCG solid source and to define relevant ranges of variation for some parameters (percentage of ethanol in the solvent and the liquid-solid ratio), before performing UAE and CE experimental designs.

3.1. Preliminary studies

3.1.1. Characterization of Spent Coffee Grounds (SCG)

The initial dry matter content of SCG was 34 ± 0.5 wt% and this value reached 96 ± 0.5 wt% after the dehydration treatment. Total polyphenols content of SCG was evaluated at 14.1 ± 1.2 mg GAE / g.dm (successive extractions were performed to exhaust SCG as described in section 2). This value is in the low range compared to those generally found in other published studies on SCG, which are in the range 17-35 mg GAE / g.dm.[27,33,34] This may be due to the storage conditions and duration until receipt and drying, and to the nature of the mix (total polyphenols content varies in function of Arabica/Robusta ratio in the mix).[35,36] The antioxidant activity of the obtained extract was evaluated at 51.0 ± 4.2 µmol Trolox / g.dm. Few published studies were performed in conditions comparable to this work and obtained values in the range 38-82 µmol Trolox / g.dm, which is in accordance with the antioxidant activity obtained in the present work.[37,38]

3.1.2. Pre-studies on two extraction parameters: ethanol content in the solvent (E) and liquid to solid ratio (R)

Many parameters could influence a solid-liquid extraction process efficiency (better final yields and/or faster diffusion of molecules), among which solid characteristics (nature, moisture content, shape, granulometry…), solvent nature, liquid to solid ratio, agitation, temperature, pressure, specific assistance (ultrasounds, microwaves, pulsed electric fields…).
Concerning SCG biowaste, it was chosen to not study particle size influence, and to use it as it was when recovered from the waste collection company. Indeed, in the perspective of a potential large scale sustainable valorization of SCG, avoiding a supplementary pre-treatment appeared suitable. The use of non-toxic solvents was favoured. To extract polyphenols from a plant matrix, a polar solvent such as ethanol, methanol or acetone must be used.[39] Here, ethanol was chosen for its low toxicity. In addition, for a big scale application, ethanol is a bio-solvent that can be generated by the alcoholic fermentation of several sugar or starch containing feedstocks, with recycling possibilities, thus suitable for the development of a sustainable process. Preliminary assays of polyphenols recovery from SCG were performed with different water-ethanol mixtures as solvent, so as to define relevant ranges of variation for the further extraction studies (design of experiments). Results presented in Figure 2A show that polyphenols content in the extract was higher with pure water than with pure ethanol. The highest polyphenols concentration (9.5 mg GAE / g.dm, corresponding to about 67% of extractible polyphenols in SCG) was reached around 50% ethanol content in the solvent. These results were in accordance with data generally observed in the literature. Ethanol content in water modifies the solubilisation of the polyphenols in the mixture by affecting the polarity and polyphenolic compounds generally have higher affinities with ethanol-water mixtures, than with pure water or pure ethanol.[40]

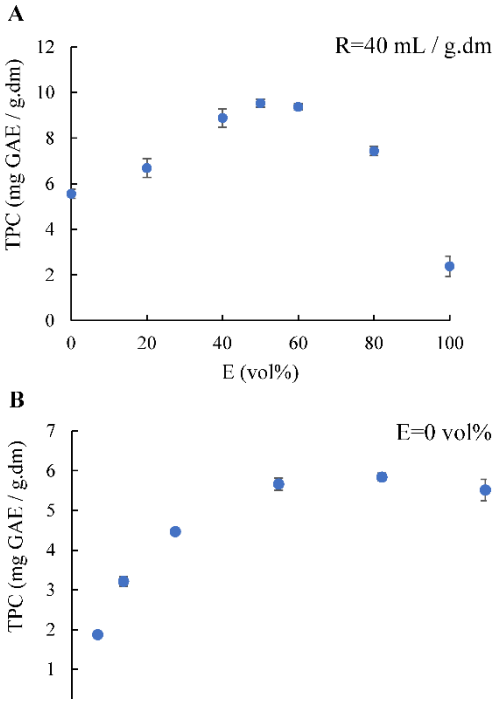

Figure 2. TPC of the extract as a function of ethanol content in the solvent E (A) and liquid to solid ratio R (B).

The range 0-50% vol% of ethanol content in water-ethanol mixtures was retained for the further studies using design of experiments. This choice allows to see if is possible to obtain good yields while trying to minimize the percentage of ethanol and make the process as green and economic as possible.
Liquid to solid ratio is an important influencing parameter on extraction efficiency. Solvent volume must be sufficient to permit a good suspension of solid phase in the liquid phase and to favour mass transfer, but not excessive to avoid solvent overconsumption and obtaining diluted extracts. Hence, preliminary assays were performed at different liquid to solid ratios, in the range 5-80 mL of solvent / g.dm of SCG (with water as solvent). TPC in the obtained extracts are shown in Figure 2B. Ratios under 20 mL / g.dm appeared clearly as too low regarding extraction performance. Maximal TPC of almost 6 mg GAE / g.dm was obtained for ratios above 40 mL / g.dm. In a concern of economic process, it was chosen not to exceed 40 mL / g.dm.

The retained range of variation for the further studies (design of experiments) was 20-40 mL / g.dm.

3.2. Comparison of Ultrasound-Assisted Extraction (UAE) and Conventional Extraction (CE) for polyphenols recovery from SCG

Process duration is a key point while studying sustainable processes. The objective is to find the right balance to obtain a high extraction yield while controlling energy consumption. To define the most relevant process duration for further UAE and CE experimental designs, it was decided to study extraction kinetics.

3.2.1 Kinetics studies

For kinetics studies, all extractions were carried out during 90 min at fixed liquid to solid ratio of 40 mL / g.dm, in order to obtain maximal TPC (as seen in section 3.1.2). For all other process parameters, assays were performed at extreme conditions of the experimental designs: 0 and 50 vol % ethanol content in the solvent for both UAE and CE, 50 and 400 W US power for UAE, 20 and 70 °C for CE. The obtained results are presented in Figure 3A for UAE and Figure 3B for CE. It appeared that process duration, presence of ethanol in the solvent, US application (for UAE) and heating (for CE) enabled to enhance polyphenols extraction. Under low conditions (■: 0 vol%; 50 W for UAE, 20 °C for CE), a limit plateau was reached at about 4 mg of GAE / g.dm for both UAE and CE. The use of a 50 vol% ethanolic solvent enabled to almost double polyphenols recovery, reaching around 8 mg of GAE / g.dm for both UAE and CE (■: 50 vol%, 50 W for UAE, 20 °C for CE). In fact, addition of ethanol in the solvent increases the solubility of phenolic compounds significantly, as seen in section 3.1.2. Then applying in addition maximal US power for UAE or maximal heating for CE (○: 50 vol%, 400W for UAE, 70 °C for CE), enabled to attain the higher TPC yields: about 12.0 mg GAE / g.dm for UAE and about 10.3 mg GAE / g.dm for CE (at 90 min of extraction). This corresponded to about 3 and 2,4 times increase compared to low conditions, and to about 85% and 73% of available TPC in the source, respectively for UAE and CE.

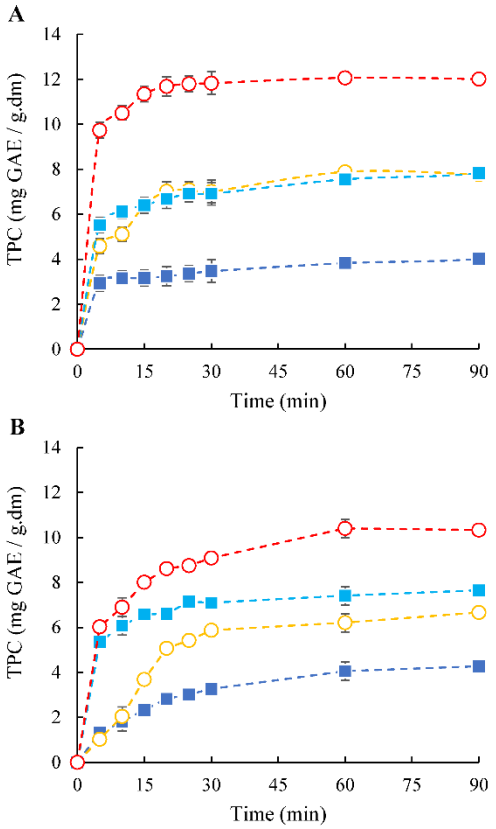

Figure 3. Kinetics of TPC during UAE (A) and CE (B) for different operating conditions. in red circles (○): 50 vol%, 400W for UAE, 70 °C for CE; in yellow circles (○): 0 vol%; 400 W for UAE, 70 °C for CE; in light blue squares (■): 50 vol%, 50 W for UAE, 20 °C for CE; in dark blue squares (■): 0 vol%, 50 W for UAE, 20 °C for CE.

Regarding kinetics considerations, maximal TPC (extraction plateau) was generally reached faster with UAE than with CE. High amounts of polyphenols were recovered in the first 5 minutes of extraction in the case of UAE. In high conditions, maximal TPC was reached in about 20-30 min for UAE, whereas it took about 60 min for CE. Indeed, US application permitted a faster heating of the solvent mixture than for CE. Ultrasounds provoke cavitation phenomena in the medium, namely the formation of bubbles which undergo compression and decompression. The repeated deformation of these bubbles lead to their explosion when the surface tension force can no longer maintain them. This leads to an increasement of the temperature and a degradation of the solid matrix, which can make the wanted extractables more accessible.[18,41,42] The raise of temperature contributes to the diffusion of molecules in the medium by lowering the viscosity and increasing the solubility. A high-power level allows higher and faster temperature raise, but it can lead to degrading part of the extractables in some cases, in particular for thermosensitive molecules.[43] With the experimental conditions used in the present study, no degradation of polyphenols was observed.
About 85% of the maximal TPC yield was reached after 30 min of extraction for all tested conditions, which might correspond to the progressive stagnation of polyphenols diffusion.

Recovering the small part of polyphenols remaining in the source, involved a much longer process duration and greater consumption of energy. Moreover, at 30 min of extraction, solvent temperature was stabilized, both for UAE or CE. Hence, it was decided to fix process duration at 30 min for further studies with experimental designs.

3.2.2 Optimization and comparison of UAE and CE

Optimization and comparison studies of UAE and CE for polyphenols recovery from SCG were performed using two designs of experiments as described in section 2.5. The objective was to compare UAE and CE performances in terms of polyphenols recovery, antioxidant activity and energy consumption. Results obtained after modelling and statistical analysis of both experimental designs are presented in Tables 3 and 4 (observed and model response for each assay), Figure 4 (surface responses and 2D graphs) and Table 5 (model coefficients values and ANOVA results). Surface responses and 2D graphs for UAE and CE are presented side by side on Figure 4 to facilitate the comparison between the two processes. Three responses are presented, polyphenols recovery (TPC), energy consumption (EC) and solvent temperature (ST), in function of varied process parameters, at 30 min of extraction. To avoid overloading of the figure with duplicate information, it was chosen not to present antioxidant activity (AA) surface response, as results were very similar to TPC. In fact, a strong concordance between TPC and AA results was observed for all assays of both UAE and CE experimental designs (at 30 min of extraction, correlation coefficients were about 0.86 and 0.90 for experimental and model result respectively). AA results are however provided in Tables 3 and 4. All R^2 and adjusted R^2 values were greater or equal to 0.90, and even close to 1 in majority (Table 5), indicating that observed and model results were in good accordance and there was no overestimation of the model due to the number of variables.

The most predictable responses were the energy consumption and the solvent temperature. Indeed, the main independent variable on which they depend is the power of the ultrasound for UAE and the water bath temperature for CE.

2D representations for both of these responses were more appropriate. For TPC, R^2 and adjusted R^2 values were lower than for the other answers, but still good enough to provides a good fit of the model to experimental data. Predicted R^2 values, indicate that the model may lack precision on the predictions over the studied domain but the values remain acceptable to conclude on trends.[44] Experimental design for UAE study was performed at first. Liquid to solid ratio (R) and ethanol content in the solvent (E) were varied in the ranges 20-40 mL /g.dm and 0-50 vol% respectively, as established in pre-studies (section 3.1.2). US power (P) was varied up to 400 W (maximum power supplied by the US equipment). Statistical analysis of the data (Table 5) revealed a significant influence of these three independent variables (p-Value < 0.05) for TPC response. US power (P) and ethanol content in the solvent (E) were the most significantly influential parameters, as observed on surface responses (Figure 4) and confirmed by model coefficient values and associated p-Values (b_1=1.28, b_3=1.69, p-Values both under 0.001). Liquid to solid ratio (R) influence was significant but less strong (b_2=1.09, p-Value under 0.01). Among interactions and quadratic terms, the only one significantly influent was the interaction between P and R (b_{12}=0.69, p-Value of about 0.01). Observing TPC surface responses for UAE (Figure 4), the effects of P and E parameters were in accordance to those obtained in pre-studies (section 3.2.A): increasing US power and ethanol content in the solvent allowed higher polyphenols recovery, with a factor of up to 3 between low and high conditions. Concerning liquid to solid ratio (R), its effect was more marked at high US power: when rising R from 20 to 40 mL / g.dm, TPC. recovery was enhanced by a factor of about 1.6 at P=400 W, whereas it was limited to about 1,2 at P=50 W. TPC yield was enhanced all along R variation range (no plateau), so that no optimal value under 40 mL / g.dm can be found for R.

Table 3. Responses observed and predicted by the model at 30 min of extraction for the 17 assays of UAE experimental design

Assay n°	Varied parameters (Coded values)			TPC mg GAE / g.dm		AA µmol Trolox / g.dm		EC W.h		ST °C	
	P	R	E	observed	predicted	observed	predicted	observed	predicted	observed	predicted
1	-1	-1	-1	2.81	3.29	2.85	3.38	60	62	32.6	31.8
2	-1	1	-1	3.47	3.34	3.80	1.73	60	57	31.8	30.6
3	1	-1	-1	5.11	4.54	10.24	9.73	180	177	72.9	72.4
4	1	1	-1	7.01	7.39	15.29	16.80	170	172	72.6	72.9
5	-1	-1	1	6.37	6.04	22.17	20.53	60	58	32.5	32.5
6	-1	1	1	6.91	7,54	30.46	30.84	60	63	29.8	30.2
7	1	-1	1	6.94	7.12	22.91	24.86	160	163	70.0	71.1
8	1	1	1	11.83	11.42	44.57	43.90	170	168	70.3	71.0
9	0	0	0	5.49	6.22	15.84	16.26	150	150	63.2	63.8
10	0	0	0	6.45	6.22	16.45	16.26	150	150	63.9	63.8
11	0	0	0	6.24	6.22	17.51	16.26	150	150	65.1	63.8
12	0	-1	0	5.12	5.35	15.66	15.32	150	150	65.2	65.7
13	0	1	0	8.00	7.53	23.17	24.01	150	150	65.2	65
14	-1	0	0	5.01	4.36	5.01	7.81	60	60	26.2	28.2
15	1	0	0	6.51	6.93	19.81	17.52	170	170	70.5	68.9
16	0	0	-1	5.15	4.99	7.57	8.11	150	152	63.4	65.6
17	0	0	1	8.45	8.37	30.27	30.23	150	148	66.6	64.8

Table 4. Responses observed and predicted by the model at 30 min of extraction for the 10 assays of CE experimental design

Assay n°	Varied parameters (coded values)		TPC mg GAE / g.dm		AA µmol Trolox / g.dm		EC W.h		ST °C	
	T	E	observed	predicted	observed	predicted	observed	predicted	observed	predicted
1	-1	-1	3.27	3.35	12.28	14.01	0	0	20.7	20.4
2	-1	1	7.10	7.12	34.98	34.43	0	0	20.5	20.5
3	1	-1	5.87	5.54	20.36	21.96	600	607	67.4	67.6
4	1	1	9.08	8.79	39.69	39.01	630	635	67.5	67.4
5	0	0	7.16	6.73	20.44	22.75	360	365	44.8	45.0
6	0	0	6.92	6.73	22.95	22.75	360	365	44.9	45.0
7	-1	0	5.00	5.00	21.91	20.72	0	0	20.5	20.2
8	1	0	6.35	6.98	27.91	26.98	640	627	67.3	67.3
9	0	-1	4.79	5.13	20.22	16.89	360	353	45.8	45.3
10	0	1	8.41	8.69	34.40	35.62	370	367	45.0	45.2

Finally, it clearly appeared that optimal conditions to maximize polyphenols recovery with UAE process were maximal values for the three varied parameters: P at 400W, E at 50 vol%, and R at 40 mL / g.dm. As expected, and confirmed in Figure 4 and Table 5, energy consumption (EC) and solvent temperature (ST) responses strongly depend on applied US power (P) (b_1 and b_{11} coefficients values were predominant). It can be observed on Figure 4 that EC increased with US power until about 300 W, and then stabilized at about 160 W.h while US power kept increasing up to 400W. This was due to security constraints for the US equipment: temperature should not exceed 70 °C to prevent damaging of the US probes. Hence, once the limit temperature of 70 °C was attained, sonication was applied in discontinuous mode.

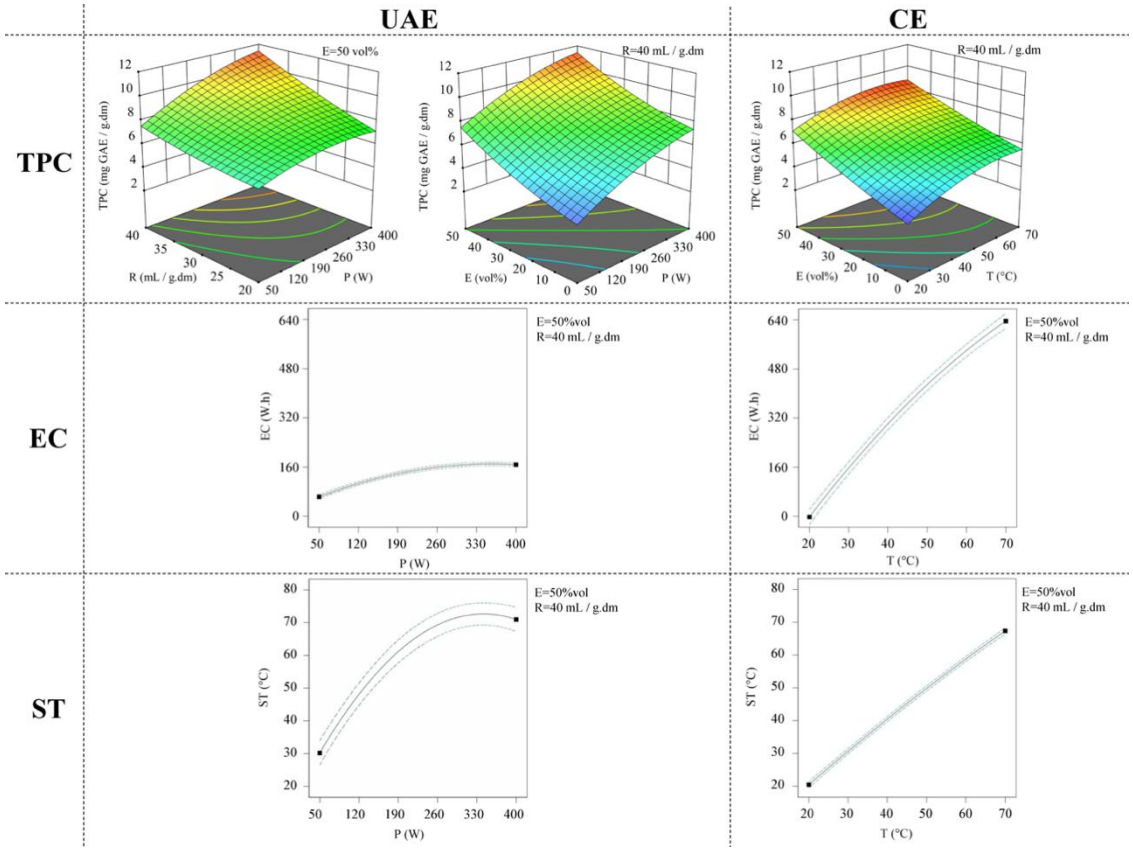

Figure 4. TPC, EC and ST responses for UAE and CE at 30 min of extraction in function of varied process parameters: liquid to solid ratio (R), US power (P) and ethanol content in the solvent (E) for UAE; temperature (T) and ethanol content in the solvent (E) for CE.

Table 5. Model coefficients and ANOVA results for both UAE and CE experimental designs

	TPC UAE		TPC CE		EC UAE		EC CE		ST UAE		ST CE	
	value	p-Value	value	p-Value	value	p-Value	value	p-Value	value	p-Value	value	p-Value
b_0	6.22		6.73		150.00		362.50		64.66		45.01	
b_1	1.28	<0.0010	0.99	<0.0100	55.00	<0.0001	311.67	<0.0001	20.34	<0.0001	23.52	<0.0001
b_2	1.09	<0.0100	1.78	<0.0010	0.00	1.0000	6.67	0.1742	-0.35	0.5493	-0.05	0.7681
b_3	1.69	<0.0001	-	-	-2.00	0.0676	-	-	-0.41	0.4851	-	-
b_{12}	0.69	0.0108	-0.15	0.5700	0.00	1.0000	7.50	0.2041	0.44	0.5046	-0.08	0.7188
b_{13}	-0.04	0.8594	-	-	-2.50	0.0464	-	-	-0.39	0.5531	-	-
b_{23}	0.36	0.1582	-	-	2.50	0.0464	-	-	-0.16	0.8014	-	-
b_{11}	-0.57	0,1905	-0.74	0.0890	-35	<0.0001	-50.00	<0.0100	-13.74	<0.0001	-1.28	<0.0100
b_{22}	0.22	0.5842	0.19	0.6030	0.00	1.0000	-5.00	0.4833	1.59	0.1838	0.25	0.4326
b_{33}	0.46	0.2770	-	-	0.00	1.0000	-	-	1.59	0.2385	-	-
R^2	0.9559		0.9627		0.9983		0.9993		0.9956		0.9998	
Adj. R^2	0.8992		0.9160		0,9961		0.9985		0,9900		0.9996	
Pred. R^2	0.4146		0.7056		0.9643		0.9939		0.9648		0.9983	

This allowed to maintain solvent temperature at about 70 °C and to generate cavitation in the mixture by intermittence until the end of extraction. Then, the second design of experiment was performed for CE, fixing the upper value of heating temperature at 70 °C to get similar temperatures in the solvent than those observed with US application at maximal power of 400W. For TPC response, statistical analysis of the data (Table 5) revealed a significant influence of the two independent varied parameters (p-Values < 0.01), the most influent being temperature (b_2=1.78), followed by ethanol

content in the solvent (b_1=0.99). Interaction and quadratic terms were not significant. For EC and ST responses, only temperature was significantly influent (only b_1 and b_{11} coefficients were highly significant), just as for UAE. Comparing UAE and CE results, the quick overall observation of 3D and the 2D graphs on Figure 4 indicates that tendencies were similar concerning parameters influence, and that CE led to less efficient polyphenols recovery and much higher energy consumption compared to UAE, when temperatures exceeded 30 °C. Temperature for CE and ultrasonic power for UAE had the same positive effect on TPC (Figure 4), both at low or high ethanol content in the solvent. But for CE, this trend began to stagnate from about 50 °C heating temperature even if the solvent temperature continues to increase. This stagnation was not found for UAE: even if the maximum solvent temperature of about 70 °C was already reached at a power of about 300 W, TPC kept on increasing until 400 W. So, it appeared that even if the temperature is a key parameter, when using ultrasound, other mechanisms such as cavitation bubbles explosions can lead to an improvement of the extraction.[45–47] The cavitation phenomena may have allowed the disintegration of the SCG agglomerates, allowing the increase of the solid-solvent contact surface and therefore the accessibility to polyphenols.[48]

For extractions carried out at low temperature for CE (20 °C) and low US power for UAE (50 W), the two studied processes showed similar efficiencies regarding polyphenols recovery (whatever ethanol content in the solvent in the range 0-50 vol%). Low level for US power was 50W, which implied a slight solvent temperature raise to about 30 °C (at 30 minutes of extraction). This did not significantly make a difference on polyphenols recovery compared to CE performed at 20 °C. This implied however a difference on energy consumption between the two processes: about 60 W.h for UAE at 50 W, against 0 W.h for CE at 20 °C. For extractions carried out at high temperature for CE (70 °C) and high US power for UAE (400 W), polyphenols recovery results were different: UAE allowed to recover about 1.3 times more polyphenols than CE (whatever the ethanol content in the solvent in the range 0-50 vol%). Then, a huge difference was observed in terms of energy consumption: about 170 W.h for UAE at 400 W, against about 640 W.h for CE at 70 °C, corresponding to a 3.7 factor difference between the 2 processes. Energy consumption and solvent temperature increased over the whole heating temperature range for CE, whereas they stagnated over about 300 W of US power for UAE. In fact, the 70 °C were attained more rapidly for UAE than CE, and then US were applied in discontinuous mode as explained above (just to maintain 70 °C), which enabled to limit energy consumption at the end of the extraction.

Finally, comparing UAE and CE results, ethanol content in the solvent was the most influencing parameter on polyphenols recovery whether for UAE or CE, followed by US power for UAE or heating temperature for CE. Liquid to solid ratio parameter was included in UAE study to possibly detect an optimal value under 40 mL / g.dm, but it appeared that best polyphenols recoveries required a 40 mL / g.dm ratio. For CE, gains in polyphenols recovery were quite limited beyond 50 °C of heating temperature, so it appeared not useful to heat at 70 °C, which would generate useless energy consumption. Hence, optimal operating conditions for CE in the studied domain were 50 vol% ethanol content in the solvent and about 50 °C heating temperature (for R=40 mL / g.dm), enabling to recover almost 9 mg GAE / g.dm TPC (about 64% of extractable polyphenols), consuming about 360 W.h of energy. For UAE, maximal polyphenols recovery of almost 12 mg GAE / g.dm was attained (about 85% of extractable polyphenols) with 50 vol% ethanol content in the solvent, US power at 400 W and liquid to solid ratio at 40 mL / g.dm, requiring about 170 W.h energy consumption. Hence, the use of ultrasounds allowed about 33% enhancement of polyphenols recovery and more than 50% energy savings compared to optimal conventional extraction in the studied domain. This might be explained by US technology advantages regarding heating of extraction mixture efficiency (compared to conventional extraction equipment with double jacket and water bath), and cavitation phenomena provoking destructuration of solid matter and thus facilitating access to extractables.[41,42]

4. Conclusion

Ultrasound-assisted extraction was demonstrated to be an efficient method to recover polyphenols from spent coffee grounds compared to conventional extraction process, both in terms of extraction yield and energy consumption. Preliminary studies and experimental designs enabled to define to most influential parameters and the optimal operating conditions for ultrasound-assisted extraction (UAE) and conventional extraction (CE) processes. Polyphenols recovery efficiency was influenced mostly by ethanol content in the solvent (polarity of the solvent), then US power in the case of UAE and heating temperature in the case of CE. Comparing UAE and CE, each one at optimized operating conditions in the studied domain, US application allowed about 33% enhancement of polyphenols recovery and more than 50% energy savings. Benefits of ultrasounds application might be explained by efficient heating of the medium (compared to CE) and cavitation phenomena facilitating access to polyphenols in spent coffee grounds (degradation of the solid matrix). These biowastes are widely available and their use is still too little promoted with solutions on an industrial scale. Produced extracts exhibited antioxidant activity that could be of interest for application in cosmetic, food and pharmaceutical areas. Large scale production of antioxidant extracts using UAE technology however implies dealing with scaling-up issues that might arise with US technology. Then, even after extraction of polyphenols, spent coffee grounds remain rich in many other compounds such as sugars, which could also be valorized in the framework of a biorefinery approach.

Author Contributions

MB performed experimentations, made the analyses and wrote the manuscript with support from PV and DP. AA, VP, PV and DP reviewed the paper. VP supervised the project.

Conflicts of interest

The authors declare no conflicts of interest.

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