Perugia, 16 e 17 aprile 2020, Università degli Studi di Perugia

XX Congresso Nazionale CIRIAF

Sviluppo Sostenibile Tutela dell'Ambiente e della Salute Umana

Circular economy and integrated supply chains for exploitation of the waste biomass coffee silverskin to valuable antioxidant polyphenols

Serena Ciorba, Domenico Licursi, Anna Maria Raspolli Galletti and Claudia Antonetti*

Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124, Pisa, Italy.

* Author to whom correspondence should be addressed. E-Mail: claudia.antonetti@unipi.it

Abstract: Coffee is one of the largest consumed drinks all over the world generating, every year, a very significant amount of wastes during the beans processing. Among these by-products, coffee silverskin is a very interesting waste product deriving from the roasting process. Today, it has been estimated that 0.8 tons of coffee silverskin are produced on 100 tons of final coffee. Up to now, these waste residues have no specific use, being mostly discharged in landfill or burned. On the other hand, in recent years, coffee silverskin has attracted great attention as promising source of bioactive compounds, including polyphenols and caffeine. However, in order to valorize these valuable components, they should be isolated from the starting matrix through suitable processes and, in this regard, recently, several extraction methods with different complexity, cost and yield have been applied. Among them, microwave-assisted extraction results particularly efficient, nowadays garnered increasing interest in various fields. On this basis, the aim of the present research is the investigation and the optimization of the solid-liquid extraction of valuable antioxidant polyphenols from coffee silverskin, under microwave irradiation. The influence of the main reaction parameters, such as temperature, extraction time and composition of the solvent, consisting of a binary mixture of water/ethanol, has been assessed, also employing statistical modeling. The achieved polyphenols yields are higher than those reported in the literature, thus leading to an important step forward, in the perspective of improving this waste recycling in a circular economy perspective.

Keywords: waste biomass, coffee silverskin, solid-liquid extraction, polyphenols, caffeine, 5-O-caffeoylquinic acid, antioxidant activity, microwaves, recycling, circular economy.

ISBN (online PDF) 978-88-9392-190-9
Published by Morlacchi Editore University Press.

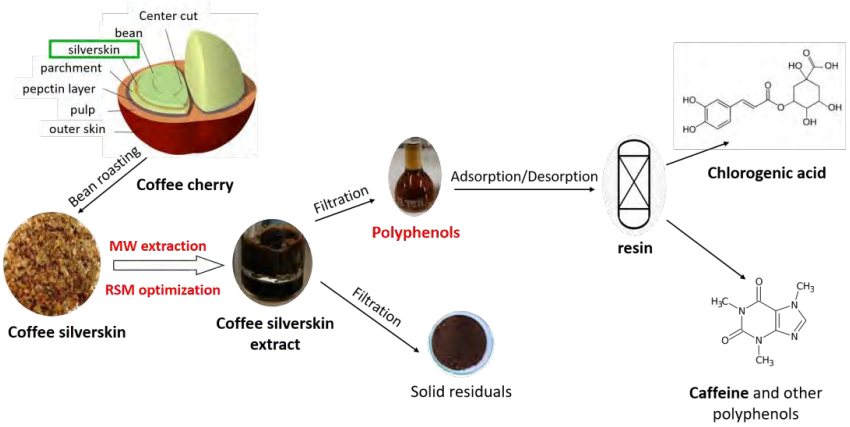
1. Introduction

Coffee is one of the most consumed drinks all over the world after water and the most commercialized product, secondly to only petroleum. According to the International Coffee Organization, Italians consume an average of 5.9 kg of coffee per capita per year and the countries of northern Europe have an even higher consumption of about 12 kg [1]. Annual world coffee exports amount to 8-10 million tons and represent a fundamental economical income for many tropical and subtropical countries. However, the processing of coffee generates massive amounts of by-products, such as husks, coffee silverskin (CS) and spent coffee, which are generally disposed as waste materials. In particular, husk is the outer coating of the coffee cherry and the main by-product of the agro-industry, while CS, which is a thin tegument that covers the outer layer of the green coffee bean, is removed during the roasting process, and, lastly, spent coffee grounds remain as solid residue after coffee brewing. For every 100 tons of final product, 200 tons of husks, 60 tons of spent coffee and 0.8 tons of CS are produced [2-3]. The large quantities of those residuals are a major problem for the manufacturers of this industrial area, both for disposal costs and for environmental issues. Nevertheless, such by-products have an interesting bulk chemical composition, being rich in nutritional elements and bioactive components, which could be valorized for new value-added applications. In addition, the European waste management policy is aimed to minimize the production of wastage or to re-use it, rather than landfill disposal [4]. Moreover, the new concepts of circular economy and biorefinery encourage the valorization of each biomass component, developing more sustainable approaches. Among all the possible coffee by-products, CS is a relatively dry feedstock due to its low moisture content (5-9 wt%) and it is generally used for the production of bioenergy, compost and organic fertilizers [5]. However, such an approach is limiting and does not fully exploit the components of this biomass. Taking into account that the exact chemical composition of CS depends on the coffee species, the geographical origins and the roasting process of the starting beans, its main components are dietary fibers about 56-65 wt% (including lignin (10-20 wt%) and carbohydrates (cellulose: 10-23 wt% and hemicellulose: 10-17 wt%)), proteins around 16-20 wt%, fats in the range 1-3 wt% and ash between 7-10 wt% [6-8]. In addition, CS contains antioxidant compounds, such as caffeine about 0.8-3 wt%, caffeoylquinic acids up to 6 wt%, melanoidins around 10-23 wt% originating as a consequence of the coffee roasting and vitamin E about 0.4 wt% [9]. Exploiting the nutritional and chemical composition, CS emerges as a highly interesting product to be used in nutraceutical, cosmetic and pharmaceutical industries [9]. Recent researches have demonstrated the anti-aging skin activity of CS extracts, particularly promising for cosmetic applications [9]. Tests *in vitro* have shown an inhibitory effect of hyaluronidase, which is responsible for hyaluronic acid degradation [9]. This last process is detrimental for human skin, which shows tendency to become dry and wrinkled, whereas CS-based creams improve skin hydration and firmness. In another investigation, it has been observed that the roundworm nematodes C. *elegans,* under short-wavelength irradiation (UV-C), increase longevity, when treated with CS extract [10]. In addition, nanostructured lipid carriers, associated with caffeine extracted from CS, can penetrate the skin barrier and act as anti-cellulite agents. CS extracts are also effective antimicrobial agents against pathogenic bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Klebsiella pneumoniae*) [9]. Moreover, CS extract inhibits the

ISBN (online PDF) 978-88-9392-190-9 -
Published by Morlacchi Editore University Press.

formation of advanced glycation products, a process that plays an important role in the development of type 2 diabetes [11]. The biological activities of the CS extracts are mainly ascribed to the contribute of the phenolic components, in particular caffeoylquinic acids, known as chlorogenic acids, and caffeine [12]. In this regard, many studies on the extraction and characterization of phenolic compounds of CS source have been carried out on laboratory-scale [13-16]. However, pilot production systems have not been developed yet, essentially due to the difficulties of obtaining a profitable extraction process from CS, achieving highly concentrated products. Conventional methods, based on solid-liquid extraction, require long extraction times, up to 3h [13] or even 18h [17], and high amounts of solvents, with CS/solvent ratios between 1/20 and 1/50 g/mL, adopting temperatures in the range 65-80°C, which make these procedures significantly energy-demanding. Other methods, implying harsher conditions, have been applied for CS, such as subcritical water extraction at 180-210°C for 10 minutes [18] and hydrothermal pre-treatment at 120°C for 20 minutes [13]. Recently, the research towards new eco-friendly, energy-efficient and effective techniques, is increasing, in order to improve the quality of the extract and reduce time and volume of solvents. In this work, the microwave (MW)-assisted extraction of polyphenols has been proposed, where the main reaction parameters, temperature, reaction time and solvent composition, have been optimized by Response Surface Methodology (RSM). MW technology has already been applied successfully to the extraction of bioactive compounds and the production of important platform chemicals from various food and lignocellulosic matrices [19-21]. RSM has been used for improving and optimizing various processes, including biomass treatments [22-25]. It uses a model equation, considering different variables at one time, fits the experimental data and processes the optimal response of the system with a limited number of experiments. Moreover, preliminary and ongoing tests have shown that the CS extracts can be treated by ion-exchange resins in order to concentrate and separate chlorogenic acids from caffeine and obtain two different fractions with specific applications, one rich in caffeine and the other one in clorogenic acids. Indeed, recently, absorption and ion-exchange processes have been widely applied for the recovery and purification of phenolic compounds from natural extracts, being techniques that can be easily realized with low-cost and low-environmental impact systems [26-27]. The smart proposed approach, outlined in Figure 1, enables us to achieve a higher concentration of total polyphenols than the CS extracts obtained with traditional methods, opening the way towards a more sustainable and economic exploitation of CS waste biomass.

Figure 1. Flow-chart for the production of coffee silverskin extracts.



ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

2. Materials and Methods

2.1. Materials

Coffee silverskin was provided by Grillo Pods Service s.r.l. (Leghorn, Italy) and used as received for the polyphenols extraction. Caffeine (99.5% purity), 5-O-caffeoylquinic acid (purity 95.0%), gallic acid (purity 99.0%), acetonitrile for HPLC and water for HPLC were purchased by Sigma Aldrich. Folin-Ciocalteu reagent was bought by Titolchimica. Anhydrous ethanol (99.9%), sodium carbonate (99.5%) and formic acid (99.0%) were acquired by Carlo Erba. All the solvents and chemicals were used as received.

2.2. Extraction procedure

Polyphenols extraction was carried out in the microwave reactor CEM Discover S-class System (Max Power = 300 W, 35 ml pyrex vial). In a typical experiment, the coffee silverskin/solvent ratio of 1g/10ml was used. During the MW-assisted extraction, the reaction slurry was mixed by a magnetic stirrer. At the end of the extraction, the mixture was filtered under vacuum on a crucible, and the recovered liquid sample was filtered on a PFTE syringe filter (0.22 µm) and analyzed by high-performance liquid chromatography (HPLC)/UV-Vis, while the solid residue was dried at 105°C for 24h, weighted and analyzed by FT-IR.

2.3. Total phenolic content (TPC)

The total phenolic content (TPC) of liquid samples was measured by the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE), as reported by Alves [28]. Briefly, in a 50 ml volumetric flask, 0.5 ml of CS extract were diluted 10-fold with water and mixed with 2.5 ml of Folin–Ciocalteu reagent. After 1 minute, 7.5 ml of sodium carbonate solution (20 wt%) were added and the flask was filled up to 50 ml with water. The reaction mixture was kept in the dark for 2h and then absorbance at 750 nm was measured using a UV-Vis spectrophotometer. The calibration curve was acquired by employing commercial standard gallic acid, in the range of concentration 50-1000 mg/L. The TPC yield, expressed as mgGAE/g of starting CS, was calculated using equation (1):

$$\text{TPC Yield (mgGAE/g)} = \frac{[\text{phenols}]\left(\frac{\text{mgGAE}}{\text{g}}\right)\cdot \text{starting solvent volume (L)}}{\text{starting biomass (g)}} \quad (1)$$

2.4. Analytical instrumentation

The quantification of caffeine and 5-O-caffeoylquinic acid in the CS extracts was carried out by HPLC, using the Perkin Elmer Series 200 instrument, equipped with a UV-Vis detector. The analysis was performed at 40 °C, using the Ascentis Express RP-Amide (Supelco) column (10 cm x 2.1 mm, with a particle diameter of 2.7 µm) and the Ascentis Express RP-Amide (Supelco) pre-column (5 mm x 2.1 mm, with a particle diameter of 2.7 µm). The mobile phase was composed of water (A) and acetonitrile (B),

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

both containing 0.3% (v/v) of formic acid. The composition of the mobile phase was changed according to the following elution gradient: 0-3.75 min 100% isocratic with solvent (A); 3.75-19.50 min from 100% to 89% of solvent (A); 19.50-27.75 min from 89% to 79% of solvent (A); 27.75-44.25 min from 79% to 60% of solvent (A); 44.25-50.25 min from 60% to 37% of solvent (A); 50.25-51.00 min from 37% to 0% of solvent (A); 51.00-52.50 isocratic min at 0% of solvent (A); lastly, 52.50-64.00 min return to starting conditions of analysis. The mobile phase flow was 0.4 mL/min. with an injection volume of 20 µL. Regarding the UV-Vis detector, the wavelength of 275 nm was selected for the analysis of caffeine, while 300 nm was selected for that of 5-O-caffeoylquinic acid. The chromatograms were processed with the Chromera software. The quantitative analysis of both compounds of interest was carried out by calibration with the respective external standards, in the range 1–50 mg/L. The yield of those compounds was calculated by equations similar to Equation 1.

Determination of TPC was carried out by UV-Vis spectroscopy, adopting the JASCO V-530 spectrophotometer, as described in paragraph 2.3.

The FT-IR characterization of CS was performed with the PerkinElmer Spectrum-Two spectrophotometer, equipped with the Attenuated Total Reflectance (ATR) apparatus. The acquisition of each spectrum was provided by 12 scans, with a resolution of 8 cm^{-1}, in the wavenumber range between 4000–450 cm^{-1}.

2.5. Statistical modeling

Response Surface Methodology and Face-Centered Central Composite Design (FCCD) were employed for the reaction optimization, by maximizing the TPC yield. The independent variables for the investigated extraction were temperature, reaction time and solvent composition, as reported in Table 1. Their levels were selected based on the reported literature and preliminary experiments carried out in the laboratory. The FCCD suggested a matrix of 17 experimental runs inclusive of 2 replicates to ascertain the significance of selected process variables and the interaction between the independent variables in order to maximize TPC yield (Table 1). Design Expert 12 (12.0.1.0) Trial Version was adopted to analyze the results and elaborate the RSM model. The quadratic equation representing the correlation between independent variables and response(s) can be written as:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{1 \le i \le j}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2$$

where Y is the dependent variable, x_1, x_2 and x_3 indicate the independent variables, β_0 is the constant and β_i, β_{ij} and β_{ii} are respectively the linear, interaction and quadratic coefficients, of the independent variables (x_1, x_2 and x_3) on the dependent variable (Y).

3. Results and Discussion

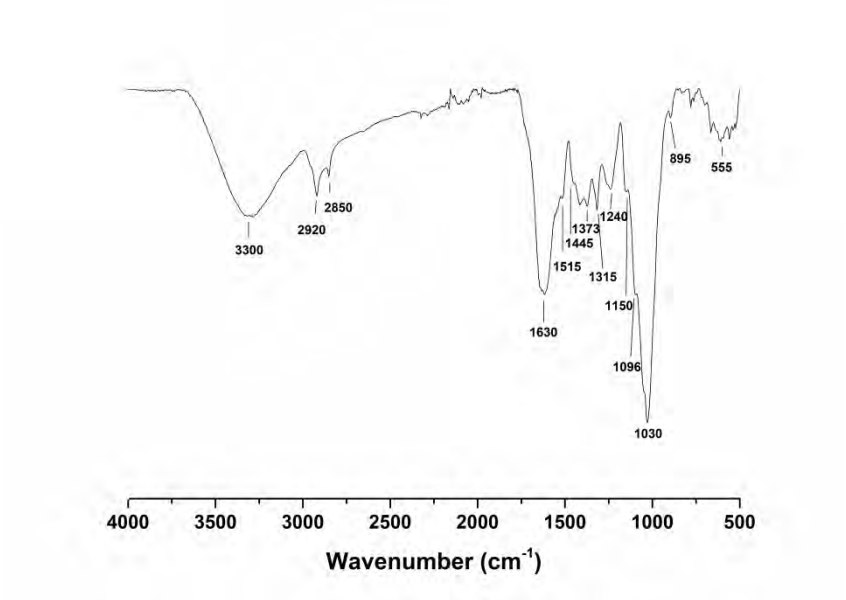
3.1. Coffee silverskin characterization

The starting CS feedstock obtained after the roasting process originates from a coffee blend, consisting of 30 wt% Arabica + 70 wt% Robusta, coming from Uganda, Brazil, Ethiopia, India and

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

Vietnam. Its moisture content was evaluated, resulting 7.5 wt%, in agreement with the literature [6]. Moreover, FT-IR analysis of the starting CS was carried out, as reported in Figure 2.

Figure 2. FT-IR spectrum of starting CS sample.



The absorption band at 3300 cm^{-1} is assigned to the stretching of the hydroxyl groups, which are typical of biomass macro-components, cellulose, hemicellulose and lignin derivatives [29]. Then, those at about 2920 and 2850 cm^{-1} are due to C-H stretching vibrations of methyl and methylene groups, which are common to all the macro-components [30]. Instead, the absorption band at 1630 cm^{-1} is related to the stretching of the C=O groups, both those conjugated to the aromatic ring of lignin and simple phenols of our interest, such as caffeine and chlorogenic acids [6,30]. The band at 1515 cm^{-1} is assigned to the deformations of the C=C bonds of the aromatic rings of lignin and simple phenols [31], whilst that at 1445 cm^{-1} is ascribed to the asymmetrical bending vibrations of the C–H bonds of the CH_2 groups of cellulose [32]. The bands at 1373 and 1315 cm^{-1} are related to the bending of the C–H and C–O bonds of the aromatic rings, respectively, whereas that at 1240 cm^{-1} to the stretching of the C–O bond of the ethers, mainly of lignin source [33]. Stretching of the C–O–C bond of the polysaccharide ring is defined by the band at 1096 cm^{-1} [34], whilst that at 1030 cm^{-1} confirms the presence of the C–OH bonds of the hydroxyl groups and the C–OR bonds of cellulose, lignin and chlorogenic acids [6,30,33]. Lastly, the small band at 895 cm^{-1} is due to the presence of β-glycosidic bonds between the monosaccharide units, while those at lower wavenumbers can be attributed to the stretching and bending deformation modes of the C–H of the aromatic ring [34]. Therefore, the FT-IR analysis of the investigated biomass qualitatively confirms its bulk lignocellulosic structure, in agreement with the literature [2,6,8].

3.2. MW-assisted solid-liquid extraction of polyphenols from CS

The solid-liquid extraction of polyphenols from CS has been investigated through the multivariate MVAT *(multi variable at time)* approach, taking into account the most noteworthy reaction variables,

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

according to the literature: temperature, reaction time and composition of the solvent, the latter composed of a binary mixture of water/ethanol. On the basis of preliminary runs, the starting biomass/solvent (g/ml) ratio has been kept constant to 1/10 (g/ml), this ratio being higher than those up to now reported in the literature, ranging from 1/20 to 1/50 (g/ml). Such a higher ratio allows a significant increase of the final concentration of the target products in the extraction solvent, both simplifying the downstream work-up operations and therefore improving the whole economy of the process [8,13]. On the basis of preliminary explorative runs, the most influential parameters were verified: temperature (T, x_1), reaction time (t, x_2) and solvent composition (%EtOH, x_3), which have been chosen as independent variables for the statistical design. The selected dependent variable, that is the response, was the total phenolic content (TPC) yield, expressed as mg of GAE for g of starting CS (mgGAE/g). Table 1 shows the operational conditions assayed in the runs of the experimental design, the independent variables and the corresponding achieved TPC yields.

Table 1. Operational conditions defining the experiments assayed for polyphenols MW-extraction from CS, in terms of dimensionless and dimensional variables and TPC yield.

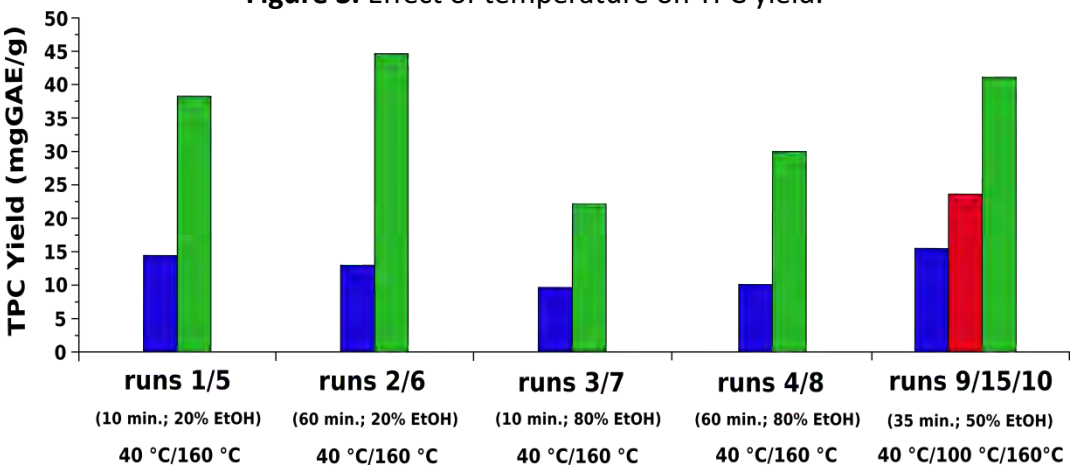
Run	Dimensionless normalized variables			Dimensional variables			TPC yield (mgGAE/g)
	x_1	x_2	x_3	Temperature (°C)	Reaction Time (min)	Solvent Composition (%EtOH v/v)	
1	-1	-1	-1	40	10	20	14.5
2	-1	1	-1	40	60	20	13.1
3	-1	-1	1	40	10	80	9.8
4	-1	1	1	40	60	80	10.1
5	1	-1	-1	160	10	20	38.3
6	1	1	-1	160	60	20	44.8
7	1	-1	1	160	10	80	22.2
8	1	1	1	160	60	80	30.1
9	-1	0	0	40	35	50	15.5
10	1	0	0	160	35	50	41.2
11	0	-1	0	100	10	50	13.3
12	0	1	0	100	60	50	21.7
13	0	0	-1	100	35	20	18.8
14	0	0	1	100	35	80	21.4
15	0	0	0	100	35	50	23.7
16	0	0	0	100	35	50	23.4
17	0	0	0	100	35	50	24.0

TPC yield ranges from the lowest value of 9.8 (run 3) to the highest one of 44.8 mgGAE/g (run 6), this last resulting higher than those up to now reported in the literature, usually lower than 35 mgGAE/g [29]. The best result reached in run 6 is very interesting, suggesting that the extraction of polyphenols is favored due to the synergy between a high temperature (160 °C) and a mainly polar solvent (20% EtOH), together with a relatively long reaction time (60 min). The beneficial effect of a

ISBN (online PDF) 978-88-9392-190-9 -
Published by Morlacchi Editore University Press.

higher temperature is clear from the data reported in Table 1 and Figure 3. In fact, comparing run 1 with 5, carried out adopting the same solvent composition (20% EtOH) and the same reaction time (10 min), different TPC yields are achieved: it increases from 14.5 to 38.3 mgGAE/g moving from 40 to 160°C, confirming the positive effect of the increase of temperature on the extraction of the target products [15,35-36]. A further evidence is observed comparing run 2 with 6, both performed using the same solvent composition (20% EtOH) and the same longer reaction time (60 min): the TPC yields are 13.1 and 44.8 mgGAE/g at 40 and 160°C, respectively, underling as the difference of TPC yields is more marked at a longer reaction time, rather than at a shorter one (compare runs 1/5 with runs 2/6). The same conclusions are drawn by comparing run 3 with 7 after 10 min of reaction and run 4 with 8 after 60 min, all experiments carried out adopting the same solvent composition (80% EtOH): also for these essays, the highest TPC yields are achieved employing the highest temperature of 160°C. In particular, the TPC yields are 9.8 and 22.2 mgGAE/g for runs 3 and 7, respectively (40 and 160°C, both 10 min), and 10.1 and 30.1 mgGAE/g for runs 4 and 8, respectively (40 and 160°C, both 60 min). Finally, the same trends are observed for runs 9, 15 and 10, which have been performed at the intermediate reaction time of 35 min and at the intermediate solvent composition of 50% EtOH, achieving TPC yields of 15.5, 23.7 and 41.2 mgGAE/g at the temperatures of 40, 100 and 160°C, respectively.

Figure 3. Effect of temperature on TPC yield.



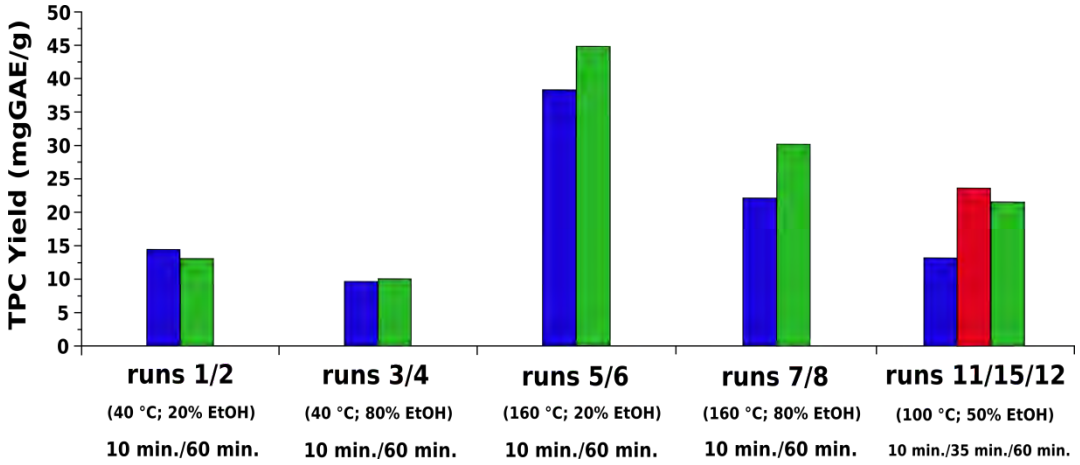
Regarding the effect of the reaction time, this variable reveals a weaker impact on TPC yield, as shown in Table 1 and Figure 4. In fact, comparing run 1 with 2 and run 3 with 4, it is possible appreciate the effect of reaction time at low temperature (40°C), with the extreme solvent compositions of 20% EtOH (runs 1 and 2) and 80% EtOH (runs 3 and 4). TPC yield remains almost constant in both cases, ranging from 14.5 to 13.1 mgGAE/g from run 1 to 2, and from 9.8 to 10.1 mgGAE/g from run 3 to 4. This scarce influence of the reaction time on TPC yield is in agreement with the literature [15]. In fact, these extraction processes usually require short reaction time to be completed, already adopting traditional heating systems, even more using the more efficient microwave heating. The employment of harsher temperature conditions, 160 °C, a favorable temperature for the extraction of polyphenols, enables us to highlight the positive effect of reaction time, adopting the two investigated solvent compositions (20% and 80% EtOH), even if to a lesser extent respect to the other variables. Comparing

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

run 5 with 6 and run 7 with 8, TPC yield, moving from 10 to 60 min, increase from 38.3 to 44.8 mgGAE/g for runs 5 and 6, respectively, and from 22.2 to 30.1 mgGAE/g for runs 7 and 8, respectively, (runs 5 and 7: 10 min; runs 6 and 8: 60 min).

A further confirmation of the short reaction time required for these extraction processes results by the comparison of runs 11, 15 and 12 where, working at the intermediate temperature of 100°C and at the intermediate solvent composition of 50% EtOH, the prolonged reaction time, from 10 to 35 and finally to 60 min, causes a considerable increase of TPC yield from 13.3 to 23.7 mgGAE/g from 10 to 35 min, whereas, after that, TPC yield remains almost constant up to 60 min (21.7 mg GAE/g).

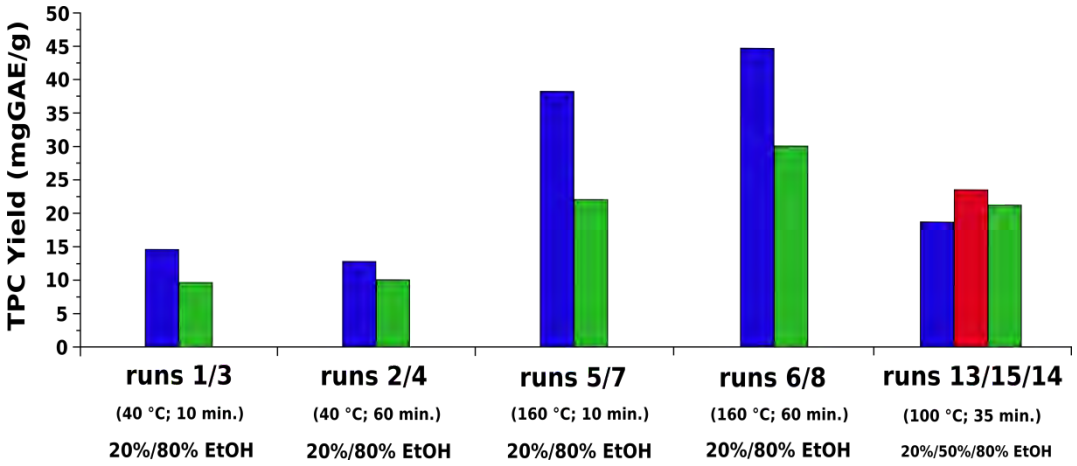
Figure 4. Effect of reaction time on TPC yield.



Finally, regarding the effect of solvent composition, reported in Table 1 and Figure 5, it is evident that polyphenols show greater compatibility with more polar solvents, such as water, rather than with ethanol, in agreement with the literature [37]. Comparing run 1 with 3 and run 2 with 4, it is possible to highlight the effect of the solvent composition at low temperature (40°C), for short (runs 1 and 3, both 10 min) and long (runs 2 and 4, both 60 min) reaction times. For both comparisons, the TPC yield increases moving from the run carried out with an excess of EtOH (80% EtOH) to the run performed with an excess of water (20% ETOH), trend observed both after 10 min (run 1 with 3) and after 60 min (run 2 with 4), being 9.8 and 14.5 mgGAE/g for runs 3 and 1, respectively, and 10.1 and 13.1 mgGAE/g for runs 4 and 2, respectively. The same observations can be inferred comparing run 5 with 7, after 10 min and run 6 with 8, after 60 min, all carried out at 160°C: at the increase of the polarity of the solvent (lower loading of EtOH), TPC yield moves from 22.2 to 38.3 mgGAE/g after 10 min and from 30.1 to 44.8 mgGAE/g after 60 min. Lastly, it is interesting to underline that when intermediate conditions of temperature (100°C) and reaction time (35 min) are employed, the effect of the solvent composition becomes negligible, achieving almost constant TPC yields of 18.8, 23.7 and 21.4 mgGAE/g, adopting 20%, 50% and 80% EtOH in runs 13, 15 and 14, respectively.

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

Figure 5. Effect of solvent composition on TPC yield.



In addition to TPC as the main response, the obtained extracts have been characterized for the content of caffeine and 5-O-caffeoylquinic acid, which are very important bioactive products for nutraceutical, cosmetic and pharmaceutical emerging applications. These yields are reported in Table 2, calculated respect to the weight of the starting CS (mg/g).

Table 2. Yields in caffeine and 5-O-caffeoylquinic acid respect to the starting CS (mg/g).

Run	Yield in caffeine (mg/g)	Yield in 5-O-caffeoylquinic acid (mg/g)
1	7.0	0.5
2	6.3	traces
3	7.4	0.3
4	7.5	0.4
5	10.0	0.5
6	12.2	0.3
7	7.1	0.3
8	7.9	0.3
9	8.4	0.4
10	8.8	0.4
11	10.3	0.8
12	10.1	0.6
13	8.1	0.4
14	7.7	0.5
15	11.9	0.7
16	11.6	1.0
17	11.9	0.7

The highest caffeine yield equal to 12.2 mg/g, is achieved in run 6 carried out at 160°C for 60 min with the solvent composition of 20% EtOH, whereas the highest 5-O-caffeoylquinic acid yield of 0.8 mg/g as average value is obtained in runs performed at the central conditions of the experimental

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

domain, runs 15/16/17 done at 100°C for 35 min with the solvent composition of 50% EtOH. These outcomes highlight as the highest caffeine yield is attained under the same reaction conditions for polyphenols, where the high temperature, the long reaction time and the great polarity of the solvent are the most favorable parameters. On the other hand, the best reaction conditions found for 5-O-caffeoylquinic acid are in agreement with its reactivity, being easily degraded through hydrolysis, isomerization and/or reactions with melanoidins [38-40]. These reactions become more significant with the increase of temperature, making necessary the use of intermediate temperatures and reaction times, together with intermediate solvent compositions (50% EtOH), considering the major affinity of this acid with such intermediate polarity.

Concerning the RSM modeling of data, Table 3 lists the values calculated for the set of regression coefficients, involved in the equations describing the behavior of the dependent variable TPC yield, as well as its statistical significance based on the Student's t-test. The same Table 3 also includes the statistical parameter R^2 measuring the correlation of the investigated model.

Table 3. Regression coefficients and the statistical parameter R^2.

Parameter	TPC yield
β_0	22.73***
β_1	11.36***
β_2	2.17*
β_3	-3.59***
β_{12}	1.94*
β_{13}	-2.89**
β_{23}	-0.39
β_{11}	6.35**
β_{22}	-4.50*
β_{33}	-1.90
R^2	0.954

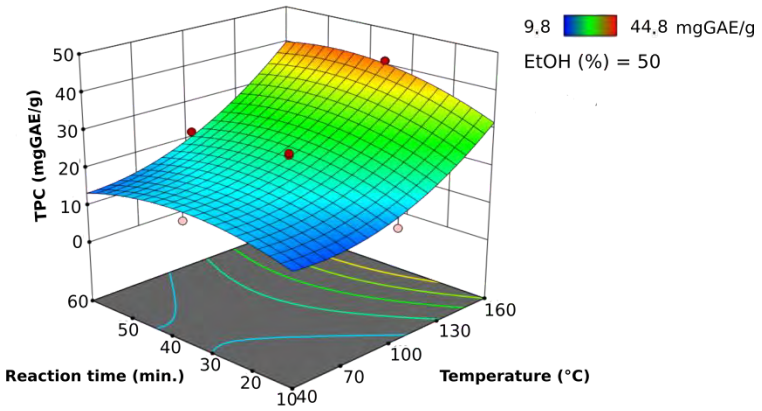
*** Coefficients significant ≥ 99% confidence level; ** Coefficients significant ≥ 95% and < 99% confidence level; * Coefficients significant ≥ 90% and < 95% confidence level.

The data reported in Table 3 show a prevailing significant dependence of TPC yield from the linear coefficients, in particular from the temperature (β_1), which represents the most relevant parameter, and, to a lesser extent, from the solvent composition (β_3), whereas the dependence from the reaction time is weak (β_2). Moreover, the positive synergy between the temperature and the solvent composition is particularly remarkable, as well as that between the temperature and the reaction time, confirmed by the β_{13} and β_{12} interaction terms. The good value of R^2 means a close agreement between the experimental results and those predicted by the model.

Figure 4 shows the calculated dependence of TPC yield on temperature and reaction time, evaluated at intermediate solvent composition (50% EtOH).

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

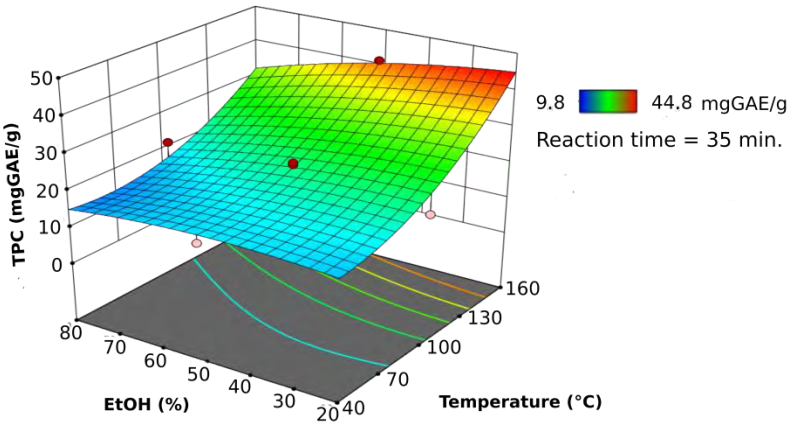
Fig. 4. Dependence of TPC yield on temperature and reaction time, calculated for intermediate solvent composition ($x_3 = 0$).



The above figure again confirms that the temperature strongly affects TPC yield, whereas the influence of reaction time is almost insignificant. At low values of temperature, in the whole investigated reaction time, TPC yield ranges between 10 and 20 mgGAE/g, whereas, in the same reaction time range but at high temperature, it is about 40-45 mgGAE/g. This is confirmed by the results of run 9 (40°C, 35 min, 50% EtOH, TPC yield: 15.5 mgGAE/g) and run 10 (160°C, 35 min, 50% EtOH, TPC yield: 41.2 mgGAE/g). Working at the intermediate temperature of 100°C, in the whole studied reaction time, TPC yield hovers between 15 and 25 mgGAE/g, as proved from run 11 (100°C, 10 min, 50% EtOH, TPC yield: 13.3 mgGAE/g), run 12 (100°C, 60 min, 50% EtOH, TPC yield: 21.7 mgGAE/g) and from runs 15/16/17 (100°C, 35 min, 50% EtOH, average TPC yield: 23.7 mgGAE/g). Employing the solvent composition of 50% EtOH, the model predicts the highest TPC yield of about 40-45 mgGAE/g at high temperature, together with long reaction time.

Figure 5 shows the calculated dependence of TPC yield on temperature and solvent composition, evaluated at intermediate reaction time (35 min).

Fig. 5. Dependence of TPC yield on temperature and solvent composition, calculated for intermediate reaction time ($x_2 = 0$).



ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

The obtained surface shows that at low temperature, in the whole explored solvent composition, TPC yield is about 15 mgGAE/g, as evidenced from run 9 (40°C, 35 min, 50% EtOH, TPC yield: 15.5 mgGAE/g). Working at the intermediate temperature of 100°C, in the whole investigated solvent composition range, TPC yield is around 20 mgGAE/g, as verified from run 13 (100°C, 35 min, 20% EtOH, TPC yield: 18.8 mgGAE/g), run 14 (100°C, 35 min, 80% EtOH, TPC yield: 21.4 mgGAE/g) and runs 15/16/17 (100°C, 35 min, 50% EtOH, average TPC yield: 23.7 mgGAE/g). Finally, when a high temperature is employed, in the whole studied solvent composition range, TPC yield ranges from 30 to 45 mgGAE/g, as confirmed from run 10 (160°C, 35 min, 50% EtOH, TPC yield: 41.2 mgGAE/g). In this case, employing the reaction time of 35 min, the highest predicted TPC is approximately 40-45 mgGAE/g, being achievable at high temperature and employing a solvent with a low EtOH concentration. Of course, in both cases of intermediate solvent composition (Figure 4) and intermediate reaction time (Figure 5), it is imperative to not increase the temperature beyond a certain limit higher than 160°C which may prop up the decomposition and/or degradation of phenolic compounds.

Figure 6 shows the calculated dependence of TPC yield on reaction time and solvent composition, evaluated at intermediate temperature (100 °C).

Fig. 6. Dependence of TPC yield on reaction time and solvent composition, calculated for intermediate temperature ($x_1 = 0$).

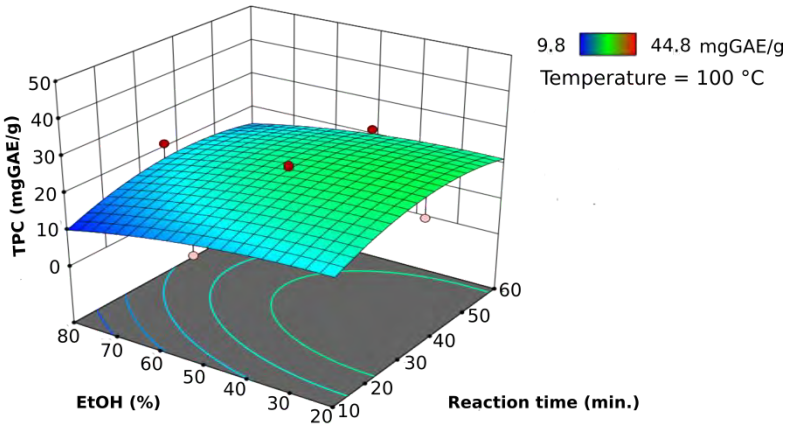


Figure 6 shows that at short reaction time, in the whole range of solvent composition, TPC yield is between 10 and 15 mgGAE/g, in agreement with the results of run 11 (100°C, 10 min, 50% EtOH, TPC yield: 13.3 mgGAE/g). On the other hand, at long reaction time, in the whole range of solvent composition, TPC yield hovers from 10 to 20 mgGAE/g, as evidenced from run 12 (100°C, 60 min, 50% EtOH, TPC yield: 21.7 mgGAE/g). Finally, working at the intermediate reaction time of 35 min, in the whole investigated solvent composition, TPC yield ranges from 15 to 25 mgGAE/g, as found in run 13 (100°C, 35 min, 20% EtOH, TPC yield: 18.8 mgGAE/g), run 14 (100°C, 35 min, 80% EtOH, TPC yield: 21.4 mgGAE/g) and runs 15/16/17 (100°C, 35 min, 50% EtOH, average TPC yield: 23.7 mgGAE/g). Working at 100°C, the obtained surface evidences the highest TPC yield about 20-25 mgGAE/g at reaction time longer than 35 min together with a solvent with low EtOH loading, less than 50% EtOH.

ISBN (online PDF) 978-88-9392-190-9 -
Published by Morlacchi Editore University Press.

The theoretical model predicts the maximal TPC yield of 45.2 mgGAE/g in the optimal conditions of 160°C for 53 min with the solvent composition of 20% EtOH. Under these conditions, the experimental average value of TPC yield carried out in triplicate resulted 43.1 mgGAE/g (Table 4), confirming the good prediction of the model.

Table 4. Triplicates of the run performed adopting the optimal reaction conditions predicted from the model, TPC and average TPC yields (mgGAE/g), caffeine and 5-O-caffeoylquinic acid yields (mg/g).

Run	TPC yield (mgGAE/g)	Average TPC yield (mgGAE/g)	Yield in caffeine (mg/g)	Yield in 5-O-caffeoylquinic acid (mg/g)
18	42.2	43.1	9.7	0.3
19	44.3		10.7	0.2
20	42.8		10.0	0.3

Table 4 shows that, adopting the best reaction conditions for the extraction of polyphenols, considerable amounts of caffeine and appreciable quantity of 5-O-caffeoylquinic acid can be obtained in the extracts, about 10.1 and 0.3 mg/g, respectively. Preliminary tests, still in progress, have proven that these valuable compounds can be successfully separated through adsorption/desorption on ion-exchange resins, in particular adopting both strong and weak base anion resins studying the effect of temperature, pH and initial products concentration, thus paving the way to the exploitation of each of the two compounds.

4. Conclusions

In this work, the exploitation of coffee silverskin, an abundant waste fraction deriving from the coffee production chain, was proposed. This fraction is nowadays disposed in landfills or burnt for energy recovery, whilst it contains value-added bioactive compounds, such as polyphenols, caffeine and chlorogenic acids, which could be advantageously exploited, once identified and optimized a suitable isolation procedure. In our investigation, the solid-liquid extraction of these compounds with a binary mixture of water/ethanol, assisted by the efficient microwave heating, was chosen for this purpose. The extraction procedure has been modeled by experimental design (DoE), which has allowed us to identify the optimal extraction conditions, in terms of temperature, reaction time and solvent composition, thus maximizing the content of total polyphenols in the extract. Maximum yield in total polyphenols of about 45 mgGAE/g, evaluated with respect to the starting biomass, was experimentally achieved, a higher value than those reported in the literature and in agreement with the forecasts of the proposed DoE model, which has been validated. The HPLC analysis of the extracts has enabled us to determine the content of caffeine and 5-O-caffeoylquinic acid, thus verifying the good extractability of these bio-active compounds, under the investigated extraction conditions. These polyphenols can be profitably exploited in cosmetic and nutraceutical applications and this valorization proposal represents an important step forward from the perspective of recycling and circular economy.

ISBN (online PDF) 978-88-9392-190-9 -
Published by Morlacchi Editore University Press.

Acknowledgements

The authors are grateful to the POR FESR 2014-2020 MASTERB project "Smart Manufacturing of AntioxidantS from Technological Recovery and valorization of Biomasses" (CUP: 7429.31052017.113000001) funded by Tuscany Region.

References

1. International Coffee Organization (ICO). Available online: http://www.ico.org/ (accessed on 3rd March 2020).
2. Murthy, P.S.; Naidu, M.M. Sustainable management of coffee industry byproducts and value addition: a review. *Resources, Conservation and Recycling* **2012**, *66*, 45–58.
3. Sanchez, D.A.; Cecilia, A.V. Chemical characterization of the coffee silverskin (Coffea arabica) in varieties Colombia and Caturra. *Revista Colombiana de Quimica* **2012**, *41*, 211–226.
4. Directive 2008/98/EC on waste (Waste Framework Directive). Available online: https://ec.europa.eu/environment/waste/framework/ (accessed on 3rd March 2020).
5. Carnier, R.; Berton, R.S.; Coscione, A.R.; Pires, A.M.M.; Corbo, J.Z.F. Coffee silverskin and expired coffee powder used as organic fertilizers. *Coffee Science* **2019**, *14*, 24–32.
6. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Costa, E.; Costa, S.G.C., Nunes, M.A.; Agostinho, A.A.; Santos-Silva, A.; Oliveira, M.B.P.P. Nutritional, chemical and antioxidant/pro-oxidant profiles of silverskin, a coffee roasting by-product. *Food Chemistry* **2018**, *267*, 28–35.
7. Ballesteros, L.F.; Teixeira, J.A.; Mussatto, S.I. Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and Bioprocess Technology* **2014**, *7*, 3493–3503.
8. Bessada, S.M.F.; Alves, R.C.; Costa, A.S.G.; Nunes, M.A.; Oliveira, M.B.P.P. Coffea canephora silverskin from different geographical origins: A comparative study. *Science of the Total Environment* **2018**, *645*, 1021–1028.
9. Bessada, S.M.F.; Alves, R.C.; Oliveira M.B.P.P. Coffee Silverskin: A Review on Potential Cosmetic Applications. *Cosmetics* **2018**, *5* , 5–15.
10. Iriondo-DeHond, A.; Martorell, P.; Genovés, S.; Ramón, D.; Stamatakis, K.; Fresno, M.; Molina, A.; Del Castillo, M.D. Coffee silverskin extract protects against accelerated aging caused by oxidative agents. *Molecules* **2016**, *21*, 721–734.
11. Fernandez-Gomez, B.; Nitride, C.; Ullate, M.; Mamone, G.; Ferranti, P.; Del Castillo, M.D. Inhibitors of advanced glycation end products from coffee bean roasting by-product. *European Food Research and Technology* **2018**, *244*, 1101–1110.
12. Rodrigues, F.; Nunes, M.N.; Alves, R.C.; Oliveira, M.B.P.P. Applications of recovered bioactive compounds in cosmetics and other products. In *Handbook of Coffee Processing by-Products-Sustainable Applications*, 1st ed.; Galanakis, C.M., Eds.; Academic Press: United Stated, 2017; Volume 7, pp. 195–220.
13. Conde, T.; Mussatto, S.I. Isolation of polyphenols from spent coffee grounds and silverskin by mild hydrothermal pretreatment. *Preparative Biochemistry and Biotechnology* **2016**, *46*, 406–409.

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

14. Tanggugh, P.; Kusumocahyo, S.P. Extraction of coffee silverskin to convert waste into a source of antioxidant. *AIP Conference Proceedings* **2017**, *1803*, 020029.
15. Guglielmetti, A.; D'ignoti, V.; Ghirardello, D.; Belviso, S.; Zeppa, G. Optimisation of Ultrasound and Microwave-Assisted Extraction of Caffeoylquinic Acids and Caffeine from Coffee silverskin Using Response Surface Methodology. *Italian Journal of Food Science* **2017**, *29*, 409–423.
16. Barbosa-Pereira, L.; Guglielmetti, A.; Zeppa, G. Pulsed Electric Field Assisted Extraction of Bioactive Compounds from Cocoa Bean Shell and Coffee Silverskin. *Food and Bioprocess Technology* **2018**, *11*, 818–835.
17. Regazzoni, L.; Saligari, F.; Marinello, C.; Rossoni, G.; Aldini, G.; Carini, M.; Orioli, M. Coffee silver skin as a source of polyphenols: High resolution mass spectrometric profiling of components and antioxidant activity. *Journal of Functional Foods* **2016**, *20*, 472–485.
18. Narita, Y.; Inouye, K. High Antioxidant Activity of Coffee Silverskin Extracts Obtained by the Treatment of Coffee Silverskin with Subcritical Water. *Food Chemistry* **2012**, *135*, 943–949.
19. Rivas, S.; Raspolli Galletti, A.M.; Antonetti, C.; Santos, V.; Parajó, J.C. Sustainable production of levulinic acid from the cellulosic fraction of *Pinus pinaster* wood: operation in aqueous media under microwave irradiation. *Journal of Wood Chemistry and Technology* **2015**, *35*, 315–324.
20. Galia, A.; Schiavo, B.; Antonetti, C.; Raspolli Galletti, A.M.; Interrante, L.; Lessi, M.; Scialdone, O.; Valenti, M.G. Autohydrolysis pretreatment of *Arundo donax*: a comparison between microwave-assisted batch and fast heating rate flow-through reaction systems. *Biotechnology for Biofuels* **2015**, *8*, 218–235.
21. Licursi, D.; Antonetti, C.; Martinelli, M.; Ribechini, E.; Zanaboni, M.; Raspolli Galletti, A.M. Monitoring/characterization of stickies contaminants coming from a papermaking plant-Toward an innovative exploitation of the screen rejects to levulinic acid. *Waste Management* **2016**, *49*, 469–482.
22. Budaraju, S.; Mallikarjunan, K.P.; Petit, R. Comparison and Optimization of solvent extraction and microwave assisted extraction of phenolic compounds from spent coffee grounds. *An ASABE Meeting Presentation* **2017**, PaperNumber: 1700127.
23. Antonetti, C.; Fulignati, S.; Licursi, D.; Raspolli Galletti, A.M. Turning point towards the sustainable production of HMF in water: metal salts for its synthesis from fructose and inulin. *ACS Sustainable Chemistry & Engineering* **2019**, *7*, 6830–6838.
24. Rivas, S.; Raspolli Galletti, A.M.; Antonetti, C.; Santos, V.; Parajó, J.C. Sustainable conversion of *Pinus pinaster* wood into biofuel precursors: a biorefinery approach. *Fuel* **2016**, *164*, 51–58.
25. Antonetti, C.; Melloni, M.; Licursi, D.; Fulignati, S.; Ribechini, E.; Rivas, S.; Parajó, J.C.; Cavani, F.; Raspolli Galletti, A.M. Microwave-assisted dehydration of fructose and inulin to HMF catalyzed by niobium and zirconium phosphate catalysts. *Applied Catalysis B: Environmental* **2017**, *206*, 364–377.
26. Kammerer, D.R.; Kammerer, J.; Carle, R. Adsorption and Ion exchange for the Recovery and Fractionation of Polyphenols: Principles and Applications. In *Polyphenols in Plants: Isolation, Purification and Extract Preparation*, 2nd ed.; Watson, R.R., Eds.; Academic Press: United States, 2019; Volume 19, pp. 327–339.

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

27. Soto, M.L.; Moure, A.; Dominguez, H.; Parajò, J.C. Recovery, concentration and purification of phenolic compounds by absorption: a review. *Journal of Food Engeneering* **2011**, *105*, 1–27.
28. Alves, R.C.; Costa, A.S.G.; Jerez, M.; Casal, S.; Sineiro, J.; Núnez, M.J.; Oliveira, M.B.P.P. Antiradical activity, phenolic profile, and hydroxymethylfurfural in espresso coffee: Influence of technological factors. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 12221–1222.
29. Alghooneh, A.; Amini, A.M.; Behrouzian, F.; Razavi, S.M.A. Characterisation of cellulose from coffee silverskin. *International Journal of Food Properties* **2016**, *20*, 2830–2843.
30. Sarasini, F.; Tirillò, J.; Zuorro, A.; Maffei, G.; Lavecchia, R.; Puglia, D.; Dominici, F.; Luzi, F.; Valente, T.; Torre, L. Recycling coffee silverskin in sustainable composites based on a poly(butylene adipate-co-terephthalate)/poly(3-hydroxybutyrate-co-3-hydroxyvalerate) matrix. *Industrial Crops and Products* **2018**, *118*, 311–320.
31. Iriondo-DeHond, A.; Ramírez, B.; Escobar, F.V.; Del Castillo, M.D. Antioxidant properties of high molecular weight compounds from coffee roasting and brewing byproducts. *Bioactive Compounds in Health and Disease*, **2019**, *2*, 48–63.
32. Di Fidio, N.; Raspolli Galletti, A.M.; Fulignati, S.; Licursi, D.; Liuzzi, F.; De Bari, I.; Antonetti, C. Multi-Step Exploitation of Raw *Arundo donax* L. for the Selective Synthesis of Second-Generation Sugars by Chemical and Biological Route. *Catalysts* **2020**, *10*, 79–101.
33. Fiore, V.; Scalici, T.; Valenza, A. Characterization of a new natural fiber from *Arundo donax* L. as potential reinforcement of polymer composites. *Carbohydrate Polymers* **2014**, *106*, 77–83.
34. Licursi, D.; Antonetti, C.; Bernardini, J.; Cinelli, P.; Coltelli, M.B.; Lazzeri, A.; Martinelli, M.; Raspolli Galletti A.M. Characterization of the *Arundo Donax* L. solid residue from hydrothermal conversion: comparison with technical lignins and application perspectives. *Industrial Crops and Products* **2015**, *76*, 1008–1024.
35. Narita, Y.; Inouye, K. Review on utilization and composition of coffee silverskin. *Food Research International* **2014**, *61*, 16-22.
36. Hadrich, B.; Dimitrov, K.; Kriaa, K. Modelling Investigation and Parameters Study of Polyphenols Extraction from Carob (*Ceratonia siliqua* L.) Using Experimental Factorial Design. *Journal of Food Processing and Preservation* **2016**, *41*, e12769.
37. Dent, M.; Uzelac, V.D.; Peni, M.; Brn, M.; Bosiljkov, T.; Levaj, B. The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts. *Food technology and biotechnology* **2013**, *51*, 84–91.
38. Sato, T.; Takahata, T.; Honma, T.; Watanabe, M.; Wagatsuma, M.; Matsuda, S.; Smith jr., R.S.; Itoh, N. Hydrothermal Extraction of Antioxidant Compounds from Green Coffee Beans and Decomposition Kinetics of 3-o-Caffeoylquinic Acid. *Industrial and Engineering Chemistry Research* **2018**, *57*, 7624–7632.
39. Xue, M.; Shi, H.; Zhang, J.; Liu, Q.Q.; Guan, J.; Zhang, J.Y.; Ma, Q. Stability and degradation of caffeoylquinic acids under different storage conditions studied by high-performance liquid chromatography with photo diode array detection and high-performance liquid chromatography with electrospray ionization collision-induced dissociation tandem mass spectrometry. *Molecules* **2016**, *21*, 948–960.

ISBN (online PDF) 978-88-9392-190-9 - © 2020 Morlacchi Editore University Press, CC BY 4.0 International.
Published by Morlacchi Editore University Press.

40. Perrone, D.; Farah, A.; Donangelo, C.M. Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 4265-4275.

ISBN (online PDF) 978-88-9392-190-9 -
Published by Morlacchi Editore University Press.