

Article

Microwave-Assisted Extraction of Bioactive Compounds and Chemical Characterisation of Coffee By-Products and Coffee

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Featured Application

Untapped, widely available biomass such as silver skin and spent grounds could be reused to extract bioactive compounds by microwave-assisted extraction, which can produce various coffee and coffee by-product extracts in a short time. By-products are rich sources of macro- and micronutrients and bioactive compounds, so their further reuse instead of disposal could be beneficial.

Abstract

This study reports the effective reuse of coffee residues from roasting and brewing processes using microwave-assisted extraction (MAE) of bioactive compounds. Principal Component Analysis (PCA) showed a significant impact ($p < 0.001$) on phenolic acid and alkaloid amounts among coffee (green and roasted) and its by-products (silver skin and spent grounds). Furthermore, a linear mixed-effects model identified that, apart from sample type, extraction temperature (50 and 70 °C) and ethanol volume fractions (50 and 70%, v/v) considerably influenced the quantities of bioactive compounds. The best conditions were achieved using 70% (v/v) ethanol at 70 °C. In addition to caffeine and chlorogenic acid, which were found in the highest amounts, the coffee and by-product samples contained high levels of total fibre (20.7–27.8%) and total fat (1.5–12.4%). Fatty acid analysis showed a dominance of oleic acid (7.55–12.7%), palmitic acid (19.3–40.3%), and linoleic acid (15.6–41.8%), with their saturated and unsaturated nature confirmed by Fourier Transform Infrared (FTIR) Spectroscopy. The reported data highlight the potential of coffee by-products as high-value sources for bioactive compound recovery.

Keywords: green coffee; roasted coffee; silver skin; spent grounds; proximate analysis; fatty acid analysis; green extraction; waste valorisation



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1. Introduction

Coffee, with its two main species, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta), is among the most traded agricultural commodities, with an estimated annual production of 174.4 million 60 kg bags, representing approximately 10.46 million metric tons per year [1]. Owing to its distinctive aroma and taste, coffee is roasted and ground to prepare

brews using hot water or steam [2]. However, before brewing, several other processing steps are undertaken, including dry, wet, semi-dried (hybrid), and post-harvest (induced fermentation) methods applied to coffee cherries to obtain green coffee (GC) [3]. Each of these processes generates a significant quantity of waste, including coffee pulp, mucilage, parchment, and husk, derived from the wet and dry methods, respectively [4]. GC beans are further processed to produce roasted coffee (RC) for consumption, and silver skin (SS), a thin outer layer of green coffee beans, is generated as a by-product of roasting. In addition to pre-consumption coffee by-products, spent coffee grounds (SG) represent a unique solid post-consumption by-product generated after preparing coffee drinks with roasted and ground coffee. All coffee pre- and post-processing by-products were mainly used for animal feed or soil composting, or otherwise ended up in landfills without further use. To prevent ecological and environmental changes caused by their decomposition in landfills, various strategies including the biorefinery concept have been proposed [4–7]. This approach offers a sustainable solution for treating coffee waste by applying innovative technologies to produce functional ingredients and nutraceuticals [3,8–10]. The presence of bioactive compounds, such as caffeine, phenolic acids, and flavonoids, as well as various macro- and micronutrients, including carbohydrates, proteins, fats, dietary fibre, minerals, and vitamins, makes coffee processing waste a promising value-added resource [3,6,9,11–13]. The main applications include nutraceutical, food, feed, and cosmetic uses [3,14,15], as well as alternative uses in microbial transformation, environmental applications, biofuels from thermochemical processes, and materials [16]. Various techniques can be used to isolate targeted analytes from coffee by-products. These include different solvents [6,13,17–19] and extraction methods. New methodologies have recently been proposed, including advanced methods such as supercritical CO₂ extraction, pressurised liquid extraction (PLE), high hydrostatic pressure extraction (HHP), MAE, and ultrasound-assisted extraction (UAE) [3,8–10,18,20–23], as these improve analyte mass transfer by weakening the cell wall. All have the same goal: to ensure high extraction efficiency while reducing extraction time, energy consumption, solvent use, environmental impact, economic cost, and waste generation. As shown in review papers, MAE [3,24,25], UAE [3,8,22] and HHP [3] have been used for spent coffee extraction. UAE, as well as PLE and MAE, have been applied for SS extraction, as reported by Urugo et al. [24] and Froisi et al. [8], respectively. Other by-products such as coffee husk and parchment have been extracted by UAE [8] and MAE [21], in addition to pulp [20,21,23]. Only two studies can be selected from the papers presented above: MAE extraction of SG [26] and SS [27]. These are used for the extraction of total phenols and fatty acids, and a few phenolic compounds, respectively, in addition to antioxidant activity testing. As previous research has examined the influence of MAE (experimentally used for process optimisation) on SG and SS, studies focusing on the extraction of individual bioactive compounds from these materials remain scarce. Furthermore, a comparison of the chemical profiles—namely proximate composition, fatty acids, and bioactive compounds, between the two groups of samples (coffee vs. coffee by-products) and their subgroups (GC, RC, SS, and SG) is not well documented in the literature. We therefore hypothesise that MAE provides a high recovery of bioactive compounds in coffee by-products, making them better sources than the coffee from which they were produced. Accordingly, the objectives of this work were: (i) to characterise and compare the proximate and fatty acid composition of GC, RC, and their by-products (SS and SG); and (ii) to evaluate the efficiency of MAE and the applied extraction parameters (solvent, temperature, and time) in recovering bioactive compounds from coffee and its by-products. The work began with proximate analysis, followed by FTIR spectroscopy of raw materials and fat extracts, fatty acid analysis using gas chromatography, and finally the application of MAE for phenolic acid and alkaloid extraction using different parameters to assess their

effect on bioactive compound quantities. Considering that coffee by-products and waste generated from roasting and brewing processes continue to increase—presenting untapped, widely available, low-cost biomass with great potential, this work provides an additional contribution to the field of coffee by-product exploitation.

2. Materials and Methods

2.1. Chemicals

All reagents, standards and solvents were of analytical grade. Sulphuric acid (pro analysi), sodium carbonate (pro analysi) and ethanol (96%) were purchased from Kefo (Zagreb, Croatia). Copper sulphate (pro analysi), potassium iodide (pro analysi), potassium sodium tartrate (pro analysi) and Folin–Ciocalteu reagent (pro analysi) were purchased from Merck KGaA (Darmstadt, Germany). All commercial standards with purity > 99% for caffeine, chlorogenic acid, caffeic acid, ferulic acid, gallic acid, *p*-coumaric acid, glucose monohydrate, bovine serum albumin, and phenol were supplied by Sigma-Aldrich (Darmstadt, Germany) and Acros Organics (Geel, Belgium), respectively. Supelco 37-component fatty acid methyl ester mix, Supelco linoleic acid methyl ester mix and Supelco linolenic acid methyl ester isomer mix were purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol (HPLC grade, >99.9%) was purchased from J.T. Baker (Philipsburg, PA, USA) and isooctane (HPLC and GC grade, >99.9%) from Fisher (Pittsburgh, PA, USA). Petroleum ether (pro analysi), dichloromethane (pro analysi), potassium bromide (pro analysi), potassium hydroxide (pro analysi), and sodium hydrogen sulphate monohydrate (pro analysi) were purchased from Kemika (Zagreb, Croatia). Formic acid and acetonitrile (HPLC grade, >99.9%) were purchased from BDH Prolabo, VWR (Lutterworth, UK). Milli-Q-grade water (Merck KGaA, Darmstadt, Germany) was used for the chemical analyses.

2.2. Coffee and Coffee By-Products

Karoma coffee beans (GC and RC) and SS were supplied by FEIO srl (Angri, Italy). SG were collected after brewing roasted coffee in the laboratory. For further information on coffee and coffee by-products, including milling, brew preparation, moisture content, and particle size distribution, refer to the work of Ninčević Grassino et al. [28,29].

2.3. Proximate Chemical Analysis of Coffee and Coffee By-Products

In addition to the moisture analysis mentioned, coffee and its by-products were evaluated for total ash, fat, and fibre content according to AOAC Official Methods [30]. All analytes were measured gravimetrically after incineration at 600 °C, Soxhlet extraction and hot digestion for 1 h with sulphuric acid and sodium hydroxide solutions, for ash, fat, and fibre, respectively. For the quantification of total sugars and proteins, a Perkin-Elmer Lambda 25 spectrophotometer (Waltham, MA, USA) was used. Before determination, samples were prepared by reflux extraction at 50 °C for 1 h, using water for sugars and a pH 7.4 phosphate buffer for proteins. Details of the procedures are provided by Ninčević Grassino et al. [31]. All measurements were conducted in triplicate, with values reported as grams of analyte per 100 g of sample.

2.4. FTIR Spectroscopy Analysis of Coffee and Coffee By-Products

In addition to proximate chemical analyses, FTIR spectroscopy was used to characterise the structure of coffee and coffee by-product crude samples. Before analysis, the samples were crushed with a mortar and pestle, mixed with dry potassium bromide, and pressed into pastilles. The fat (triglyceride) extracts obtained after Soxhlet extraction of coffee and coffee by-products were also analysed by FTIR spectroscopy. For this purpose, the resinous extracts of the oils were dissolved in dichloromethane of the highest spectral purity.

Infrared spectra in the wavelength range $4000\text{--}500\text{ cm}^{-1}$ were recorded as KBr pastilles or dichloromethane solutions using a Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a deuterated triglycine sulphate (DTGS) detector. The resolution was 4 cm^{-1} .

2.5. Determination of Fatty Acids in Fat Extracts of Coffee and Coffee By-Products

Fatty acid methyl esters (FAMEs) were prepared by transesterifying the fat extracts from coffee and its by-products. This process followed the methodology described by Petrović et al. [32] using a methanolic potassium hydroxide solution after Soxhlet extraction. Chromatographic separation was performed on a Varian CP-3800 gas chromatograph (Palo Alto, CA, Santa Clara, CA, USA) equipped with a flame ionisation detector (FID). Separation was achieved using a DB-23 capillary column ($60\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness; Agilent Technologies, Santa Clara, CA, USA). The oven programme started at $60\text{ }^{\circ}\text{C}$ (1 min), followed by a $7\text{ }^{\circ}\text{C}/\text{min}$ ramp to $220\text{ }^{\circ}\text{C}$. Helium was used as the carrier gas at a constant flow of $1.5\text{ mL}/\text{min}$. With a split ratio of 1:30 and a $1\text{ }\mu\text{L}$ injection volume, the injector and detector were set to $250\text{ }^{\circ}\text{C}$ and $280\text{ }^{\circ}\text{C}$, respectively. Data acquisition and FAME identification by retention time comparison were managed using Star GC Workstation software (Version 6.4; Varian, Palo Alto, CA, USA). All analyses were conducted in triplicate, and the results are reported as grams of fatty acid per 100 g of sample.

2.6. Microwave-Assisted Extraction of Coffee and Coffee By-Products

Microwave-assisted extraction was performed using a Milestone START S Microwave Labstation for Synthesis (Bergamo, Italy) in an open-vessel configuration. Milled samples (1.5 g) were mixed with 50 mL of solvent (50% or $70\% v/v$ ethanol) and extracted for 5 or 15 min at 50 or $70\text{ }^{\circ}\text{C}$ at 60 Hz in a round-bottom glass flask equipped with a reflux condenser to prevent solvent loss. To ensure thermal homogeneity and prevent localised overheating, the mixture was continuously stirred magnetically at a controlled speed. Microwave power was regulated by a PID (Proportional-Integral-Derivative) controller, which automatically adjusted the power (up to a maximum of 400 W) to achieve and maintain the target temperatures (50 or $70\text{ }^{\circ}\text{C}$). The temperature was monitored in real time using a contactless infrared (IR) sensor focused on the base of the flask. At the end of MAE, the samples were cooled to room temperature, filtered, and the extracts were stored at $-8\text{ }^{\circ}\text{C}$ until analysis.

2.7. Determination of Bioactive Compounds in Coffee and Coffee By-Products by UPLC-MS² Analysis

Analysis of coffee and coffee by-product samples after MAE was conducted according to our previous study [29] using an Agilent 1290 series RRLC instrument (Agilent, Santa Clara, CA, USA) integrated with a QqQ 6430 triple quadrupole mass spectrometer. Caffeine, chlorogenic acid, caffeic acid, ferulic acid, gallic acid, and *p*-coumaric acid were identified by comparing the mass spectra and fragmentation patterns with those of authentic standards, while identification of other compounds was based on previously reported mass fragmentation patterns. Quantitative data for all compounds were obtained using calibration curves of known standards. For compounds without a commercial standard, quantification was performed as follows: cafestol, kahweol, and trigonelline were quantified using the caffeine calibration curve; 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, quinic acid, and neochlorogenic acid using the chlorogenic acid calibration curve; 3-feruloylquinic acid using the ferulic acid calibration curve; 3-*p*-coumaroylquinic acid using the *p*-coumaric acid calibration curve; and 5-O-galloylquinic acid using the gallic acid calibration curve. An external standard calibration methodology was applied and calibration curves were obtained by injection of six known concentrations of authentic standards prepared by consecutive dilutions from a stock solution. The analytical method

quality parameters for chlorogenic acid, caffeic acid, ferulic acid, and gallic acid, such as the calibration curve, instrumental detection (LOD), and quantification (LOQ) limits were reported by Elez Garofulić et al. [33]. The LOD and LOQ for each analyte were determined at signal-to-noise (S/N) ratios of 3 and 10, respectively. For caffeine and *p*-coumaric acid, calibration curves were generated using six concentration levels prepared by serial dilution of stock solutions, ranging from 5 to 86.66 mg/L for caffeine and 17.5–110 mg/L for *p*-coumaric acid. Analytical parameters of the UPLC-MS² quantitative method for caffeine were as follows: regression equation $y = 3252.5x + 3927.5$, $R^2 = 0.997$, LOD = 0.0020 mg/L, and LOQ = 0.0061 mg/L. For *p*-coumaric acid: regression equation $y = 2070.68x + 4089.6$, $R^2 = 0.989$, LOD = 0.0006 mg/L, and LOQ = 0.0019 mg/L.

All analyses were conducted in triplicate and the results are expressed as mg of compounds per L of coffee and coffee by-product extract.

2.8. Statistical Analysis

All statistical analyses were conducted in R (R Core Team, version 4.1.1) [34]. Before group comparisons, the Shapiro–Wilk test was used to assess the normality of the data distribution. Appropriate parametric or non-parametric tests were selected based on the distribution characteristics of the dataset. When the data met the assumptions of normality, group comparisons (GC, RC, SS, and SG) were performed using one-way ANOVA, followed by an appropriate post hoc test (Tukey's test) to assess pairwise differences (GC–RC, GC–SS, GC–SG, RC–SS, RC–SG, and SS–SG). For comparisons between two normally distributed groups (coffee vs. coffee by-products), a Student's *t*-test was applied. When the data did not follow a normal distribution, non-parametric methods were used. For comparisons between two groups, the Mann–Whitney U test was applied. For comparisons among more than two sample types, the Kruskal–Wallis test was used as a non-parametric alternative to ANOVA. Significant Kruskal–Wallis results were followed by Dunn's post hoc test with appropriate *p*-value adjustments to determine pairwise differences. In cases where post hoc analyses did not identify statistically significant differences between groups, no specific annotations were included in the graphical representations. PCA was employed to elucidate multivariate patterns in the bioactive compound composition of coffee and coffee by-product extracts, using the FactoMineR and factoextra packages. Prior to PCA, variables were mean-centred and scaled to unit variance (scale.unit = TRUE) to ensure equal contribution of all variables and to account for differences in measurement range. Biplots were generated using fviz_pca_biplot() from factoextra, displaying sample scores and variable loadings simultaneously with 95% confidence ellipses. Variable loadings indicate the relative contribution of each variable to the principal components. The confidence ellipses were calculated at the 95% confidence level and are presented for descriptive visualisation only. PCA was used as an exploratory tool and was not intended for inferential hypothesis testing. The final visualisation was rendered using a minimalist ggplot theme for clarity. Following PCA, the individual sample coordinates for the first principal component (Dim.1), which captured the dominant variance associated with bioactive compound composition were extracted from the PCA object and used as a univariate response variable for subsequent statistical modelling. As the experimental design included both fixed factors (group, temperature, ethanol, and time) and a nested categorical structure (subgroup nested within group), the data were analysed using linear mixed-effects models (LMMs). Linear mixed-effects models (LMMs) evaluate the statistical significance of fixed factors while accounting for nested or random effects. Therefore, PCA and LMMs address different questions and may yield apparently different results. Mixed models were fitted in R using the lme4, lmerTest, and emmeans packages. The model was fitted using restricted maximum likelihood (REML), with *p*-values for fixed effects obtained using Satterthwaite's

approximation as implemented in lmerTest. Pairwise marginal means were computed using emmeans, where relevant.

Effect sizes were calculated for bioactive compounds extracted by MAE using partial eta squared (η^2_p) from three-way analysis of variance (ANOVA) models, which included temperature, ethanol concentration, extraction time, and their interactions. Analyses were performed separately for each sample type (GC, RC, SS, SG) to avoid confounding effects from matrix differences. All statistical analyses were conducted in R using the dplyr and effectsize packages.

3. Results and Discussion

This work presents the results of proximate analysis of coffee (GC and RC) and coffee by-products (SS and SG), followed by FTIR spectroscopy of crude samples and fat extracts, fatty acid analysis by GC-FID, and MAE using 50% and 70% ethanol for 5 and 15 min at 50 and 70 °C to assess their effect on the recoveries of phenolic compounds and alkaloids, as determined by UPLC-MS² chromatography. Figure 1 outlines the analyses conducted, with the main findings described in the following paragraphs.

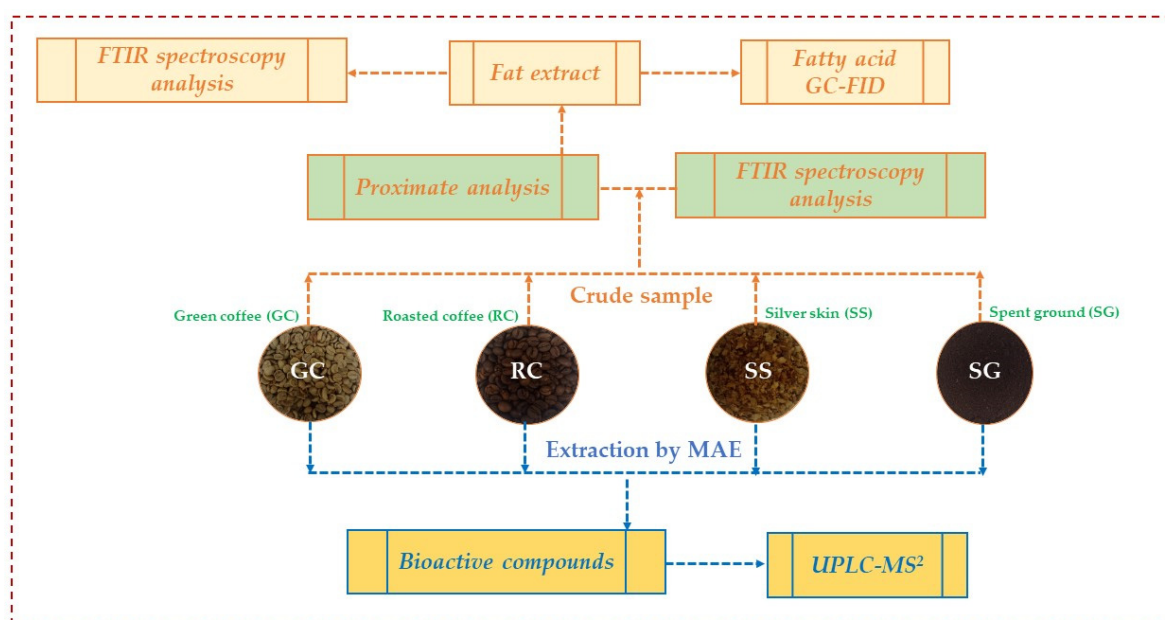


Figure 1. A schematic presentation of the analysis conducted on coffee and coffee by-products.

3.1. Chemical Characterisation of Coffee and Coffee By-Products

The results of the proximate analysis of coffee and coffee by-products (Table 1a) did not exhibit significant differences among the coffee and coffee by-product samples ($p = 0.49$), or their subgroups: GC, RC, SS, and SG ($p = 0.93$, Tukey's post hoc test, $p > 0.05$ for all pairwise comparisons), regardless of the analytes examined (moisture, ash, fat, fibre, protein, and sugar) (Table 1b).

RC had the lowest moisture content at 1.0%, followed by SS (6.2%), GC (7.2%), and SG, which tended towards the highest level at 11.9%. These results were as expected, given the typical steps in coffee production. During roasting, the application of specific temperatures and times to GC reduces its moisture content, so that by the end of processing, the roasted beans contain the least moisture. As shown by Alamri et al. [35], the moisture content in light, medium, and dark roasts of Arabica coffee is 4.8%, 4.3%, and 3.9%, respectively. Oven- and microwave-roasted coffee had 2.6% and 2.7% moisture, respectively, while GC contained 4.6% [36]. Canzi et al. [37] reported higher moisture levels for Arabica coffee beans at 8.7% compared to this work, which can be explained by species diversity. SS

contained slightly less moisture than GC, and a similar level to that reported by Gottstein et al. [38] for Arabica (6.2%) and Robusta (6.6%) varieties. Costa et al. [39] and Mota et al. [40] reported quantities of 4.8% and 8.0%, respectively, for SS samples for which the variety was not specified. Since RC is used to prepare brews with water, the moisture content in SG was expected to show a numerical trend towards higher values (11.9%) than in GC, RC, and SS. This moisture level differs from that reported by Osorio-Arias et al. [22] and Bijla et al. [41], with values of 5.3% and 18.6%, respectively, confirming differences between varieties.

Table 1. (a) Results of proximate chemical composition of coffee and its by-products. (b) Statistical data on the proximate chemical composition of coffee and its by-products.

(a)				
Analyte	Coffee		Coffee By-Product	
	Green	Roasted	Silver Skin	Spent Ground
<i>w</i> (Analyte)/%				
Moisture	7.22 ± 0.20	1.03 ± 0.08	6.20 ± 0.09	11.85 ± 0.47
Ash	4.28 ± 0.03	4.64 ± 0.07	9.89 ± 0.11	1.53 ± 0.07
Fat	12.41 ± 0.13	8.89 ± 0.85	1.50 ± 0.10	11.84 ± 0.19
Fibre	26.68 ± 0.50	24.73 ± 0.32	27.82 ± 0.81	20.72 ± 0.58
Proteins	13.68 ± 0.00	25.61 ± 0.36	8.76 ± 0.37	4.36 ± 0.26
Sugars	5.49 ± 0.03	2.02 ± 0.01	1.63 ± 0.01	0.62 ± 0.01
(b)				
Test		Statistic		<i>p</i> -value
ANOVA (GC, RC, SS and SG)		F value = 0.15, df = 3		0.93
Student's <i>t</i> -test (coffee vs. coffee by-products)		<i>t</i> = −0.68, df = 21.73		0.49

Values are means ± standard deviations of three (*n* = 3) measurements.

Ash content is very similar in both GC (4.3%) and RC (4.6%) (Table 1a), while SS showed a tendency towards a higher value (9.9%). As ash content represents the inorganic residues remaining after ashing, this indicates that SS, followed by RC and GC, would be valuable mineral sources [28] compared with SG, which has an ash level of 1.5%. Alamri et al. [35] reported values for RC ranging from 3.9% to 4.1%, depending on roasting level. SS from Arabica and Robusta contained 8.2% and 9.5%, respectively [38], and 8.3% for an unspecified variety [40]. According to Osorio-Arias et al. [22] and Mesa et al. [42], SG contained 0.35% and 2.2% ash, respectively, again confirming its low level in this by-product.

Fat content in GC was 12.4%, followed by SG at 11.8%, RC at 8.9%, and SS at 1.5% (Table 1a). These values are comparable to those reported by Canzi et al. [37] (10.9%) and Alamri et al. [35] (10.4% to 10.7%). Gottstein et al. [38] reported values of 1.5% and 1.6% for Arabica and Robusta SS varieties, which were similar to those found in this study. High fat levels in SS were reported by Iriondo-De Honda et al. [43] and Echeverri-Giraldo et al. [44], with values of 2.6% (Arabica), 3.6% (Robusta), and 12.0%, respectively. As shown in this study and other research, SG had fat levels of 10.5% [22] and 18.6% [41], making it a valuable substrate for the production of biodiesel and biogas [14].

The total fibre content remained consistently high across all samples: 20.7% (SG), 24.7% (RC), 26.7% (GC), and 27.8% (SS) (Table 1a). For RC, Alamri et al. [35] reported a fibre content of 24.3% to 28.4%, while Gottstein et al. [38] showed values of 62% for Arabica and 67% for Robusta. Osorio-Arias et al. [22] found a fibre level of 77.3% in SG, whereas for SS, reported values include 56.4% [39], 65.87% [45], and 67.7% and 69.3%, depending

on the variety [42]. Although coffee was a good source of fibre, its discharged by-products could provide an additional nutritional contribution [5,16,46].

Both GC and RC are good sources of protein and sugars, containing 13.7% and 25.6% protein, and 5.5% and 2.0% sugars, respectively, compared to their by-products (Table 1a). SG and SS contained 4.4% and 8.8% protein, and 0.6% and 1.6% sugars, respectively. Therefore, in addition to providing valuable amounts of fibre, these by-products could be effectively used as low-protein and low-sugar sources. Since SG contained a high level of moisture and fat, it can be added to products that require a certain level of hydration or fat, in contrast to SS. In each case, their functionality and safety must be ensured before use. For comparison, Alamri et al. [35], Canzi et al. [37], and Gottstein et al. [38] reported that RC contained 11.1–13.5% protein (depending on roasting degree), 15.8%, and 18.1–22.2% (depending on variety), respectively. The notable trend towards higher protein values found in this work for RC compared with GC, SS, and SG can be explained by the fact that, during roasting, degradation of carbohydrates (sucrose and polysaccharides) and formation of melanoidins occur, which are prone to interact with free amino acids and proteins, thereby increasing the total protein content. The protein content (12.3%) in SG [22], as well as that found in SS—10.9% and 19% depending on variety [43], 16.3% [45], and 18.8% [39], was higher than that found in this study. Although the sugar quantities of GC, RC, SS, and SG were not reported in the literature, differences between the amounts of analytes examined here and in other research are evident. These differences can be explained by variations in coffee variety and origin, processing conditions such as the degree of roasting and grinding of coffee beans, and brewing practices. In addition, the nature of the SG, including whether they originate from coffee shops or industrial-scale processes, also plays a role. Therefore, before using coffee and its by-products, it is necessary to provide a comprehensive chemical analysis.

3.2. FTIR Analysis of Raw and Oil Extract of Coffee and Coffee By-Products

The FTIR spectra of coffee and coffee by-product raw samples are shown in Figure 2. Similar absorption bands were observed in all four spectra (a): GC, RC, SS, and SG. A strong, broad absorption band at $3380\text{--}3390\text{ cm}^{-1}$, corresponding to the stretching vibrations of OH bonds can be primarily attributed to carbohydrates and, to a lesser extent to various organic acids such as chlorogenic acid, which is commonly found in coffee [47,48]. Two narrow bands in the range $2925\text{--}2852\text{ cm}^{-1}$, assigned to the asymmetric and symmetric stretching of methylene groups in alkyl chains (mainly from long-chain fatty acids), were also present in all four spectra. The band around 1730 cm^{-1} , corresponding to the stretching vibrations of C=O ester bonds in triglycerides [49,50] is absent only in the spectrum of SS. This is consistent with the chemical composition data for fat content in the SS sample. The strong absorption peak at approximately 1655 cm^{-1} can be attributed to the C=O carbonyl stretching of caffeine [47,48] and also to the amide I of the peptide bond in proteins. Amide II (NH stretching vibration of the peptide bond) appears as a small shoulder at approximately 1530 cm^{-1} . Additionally, the absorption band of lower intensity at $1160\text{--}1150\text{ cm}^{-1}$ can be assigned to the asymmetric stretching of C–O–C glycosidic bonds in carbohydrates (especially polysaccharides from fibres) [51] and the strong band at $1062\text{--}1056\text{ cm}^{-1}$ to the symmetric stretching of C–O–C and C–OH bonds in carbohydrates [52]. Thus, the absorption bands observed in the FTIR spectra correspond to the chemical structures of the analytes identified in the coffee and coffee by-product samples.

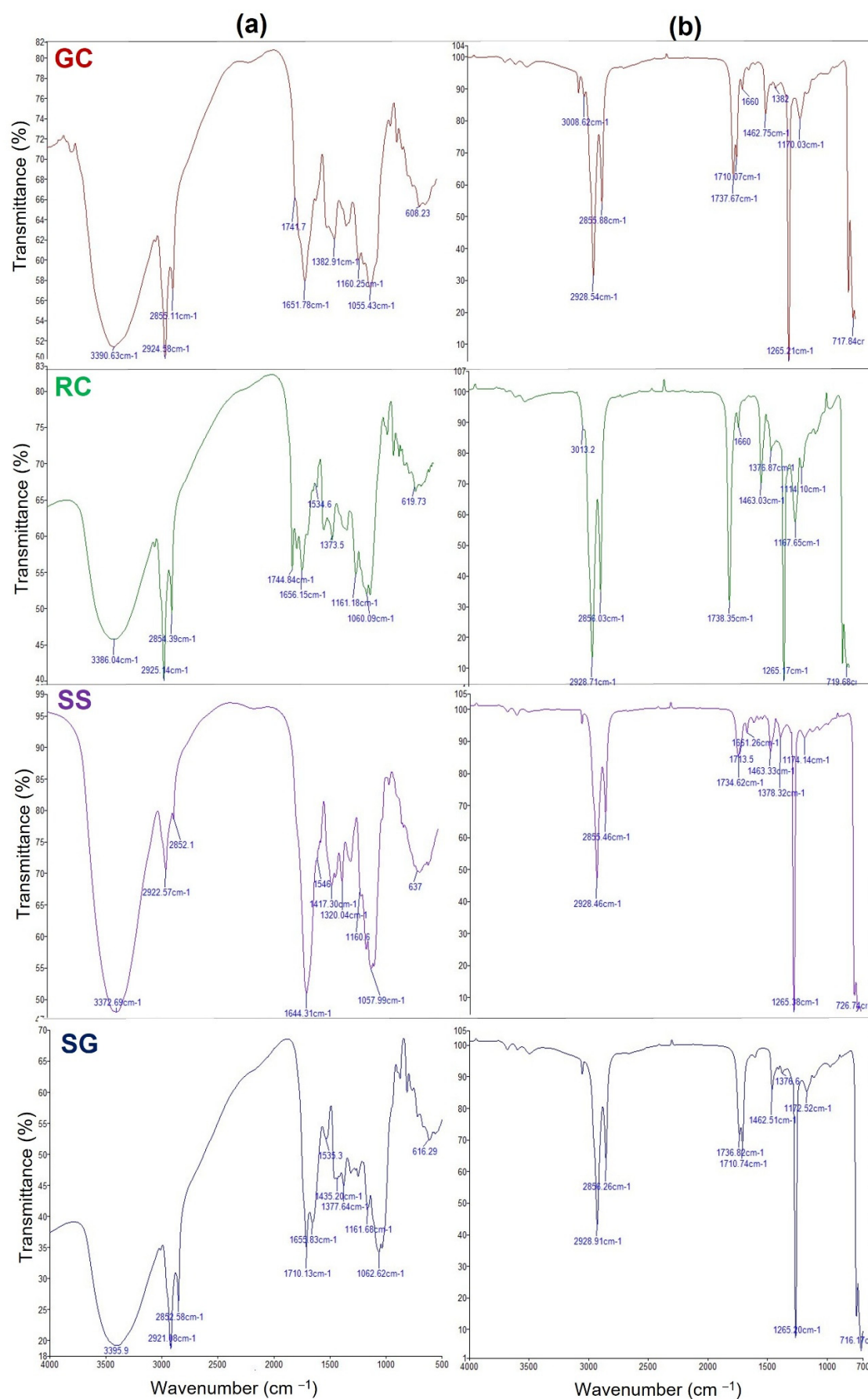


Figure 2. (a). FTIR spectra of raw coffee and coffee by-product samples. (b). FTIR spectra of their extracted oil samples. GC = Green coffee; RC = Roasted coffee; SS = Silver skin; SG = Spent ground.

The FTIR spectrum of oils can be divided into four main regions: (1) the region between 3020 and 2800 cm^{-1} , assigned to the C–H stretching vibrations of =C–H groups of unsaturated, or methyl and methylene groups of saturated lipid chains; (2) the region between 1750 and 1650 cm^{-1} , assigned to the stretching vibrations of double bonds such as C=O ester bonds of triglycerides and C=C double bonds of unsaturated fatty acids; (3) the region between 1470 and 1370 cm^{-1} , corresponding to the bending vibration of C–H bonds of aliphatic groups and disubstituted olefins; and (4) the fingerprint region between 1250 and 700 cm^{-1} , assigned to the stretching vibration of C–O ester bonds and the bending vibration of =C–H groups of the trans isomers (at approx. 965 cm^{-1}) and *cis* isomers (at approx. 723 cm^{-1}) of disubstituted olefins. The FTIR spectra of the coffee and coffee by-product oil extracts obtained by Soxhlet extraction are shown in Figure 2b. In all four spectra—GC, RC, SS and SG), two absorption bands at approx. 2928 and 2856 cm^{-1} are observed corresponding to the asymmetric and symmetric C–H stretching vibrations of the aliphatic CH_2 groups, respectively. The band of symmetric C–H stretching vibration of the *cis* double bond (=C–H) can be recognised as a small shoulder at 3009 cm^{-1} [53,54]. According to Vlahos et al. [55], oils with a high proportion of polyunsaturated acyl groups have higher frequencies at this band (ca. 3009 cm^{-1}) than oils with a high proportion of oleic acyl groups (ca. 3006 cm^{-1}), which is consistent with our findings of a high polyunsaturated fatty acid content in the oils of all four coffee and coffee by-product samples (see paragraph 3.3). The band corresponding to the stretching vibrations of the C=O ester bonds of the triglycerides is visible in all spectra at 1737–1734 cm^{-1} , and the appearance of a band at approx. 1710 cm^{-1} confirms the presence of free fatty acids [56] in all samples except the RC oil (b), in which it is absent. The small shoulder at 1660 cm^{-1} in all spectra corresponds to the stretching vibration of the C=C double bonds of *cis*-olefins [55,57]. The bending vibrations of the C–H bonds in the methylene groups are identifiable as bands of low intensity at 1464–1463 and 1378–1376 cm^{-1} , while the stretching vibrations of the C–O ester groups in triglycerides appear at 1265 cm^{-1} (very strong intensity band) and at 1172–1167 cm^{-1} (low intensity band). The out-of-plane bending vibration of the *cis* isomers of disubstituted olefins (*cis* double bond) occurs at approx. 723 cm^{-1} and is usually overlapped with a rocking vibration of CH_2 groups [54,55]. In all spectra, a strong absorption band at 720–716 cm^{-1} is observed, corresponding to the *cis* conformation of the double bonds in triglycerides, as well as a separate band at approx. 755 cm^{-1} , which can be attributed to the aliphatic CH_2 rocking vibration. In summary, the FTIR spectra of the oil samples from coffee and coffee by-products are consistent with the structure of triglycerides, which contain saturated fatty acids and *cis* conformations of the double bonds in the unsaturated fatty acids. The band of free fatty acids can also be identified in the spectra of the oil samples GC, SS and SG.

3.3. Fatty Acid Analysis of Coffee and Coffee By-Products

The results of the fatty acid analysis of coffee and its by-products are presented in Table 2a. Their profiles and quantities were compared with recent studies [36,39–41,44,58–69] summarised in Table S1 (Supplementary Materials, SM), which differ due to variety, geographic origin, extraction method, roasting type, temperature, and other factors. The results of the statistical analysis (Table 2b,c) revealed that coffee and coffee by-product samples, or their subgroups (Dunn's post hoc test for Table 2b, $p > 0.05$ for all pairwise comparisons; Tukey's post hoc test for Table 2c, $p > 0.05$ for all pairwise comparisons) are not significantly different, regardless of the levels of individual fatty acids and total fatty acids.

Table 2. (a) Fatty acid content in coffee and by-products. (b) Statistical data on the individual fatty acid content in coffee and coffee by-products. (c) Statistical data on total fatty acid content (SFA, MUFA, PUFA, UFA, omega-6, and omega-3) in coffee and coffee by-products.

(a)				
Fatty Acid	Coffee		Coffee By-Product	
	Green	Roasted	Silver Skin	Spent Ground
<i>w</i> (Fatty Acid)/%				
C16:0	40.32 ± 0.38	34.79 ± 0.14	19.33 ± 0.29	34.36 ± 0.53
C18:0	9.36 ± 2.16	7.39 ± 0.03	5.15 ± 0.06	7.65 ± 0.10
C20:0	3.57 ± 0.23	2.55 ± 0.02	18.31 ± 0.05	2.72 ± 0.02
C22:0	0.83 ± 0.23	0.41 ± 0.01	20.67 ± 0.18	0.55 ± 0.14
C23:0	0.09 ± 0.00	0.09 ± 0.00	0.52 ± 0.08	0.10 ± 0.02
C24:0	0.38 ± 0.00	0.20 ± 0.01	4.97 ± 0.02	0.26 ± 0.05
C18:1n9 <i>cis</i>	12.71 ± 1.52	11.46 ± 0.03	7.48 ± 0.47	10.15 ± 2.09
C20:1n9	0.45 ± 0.10	0.38 ± 0.01	0.24 ± 0.01	0.34 ± 0.07
C18:2n6 <i>cis</i>	25.31 ± 3.53	40.85 ± 0.19	15.55 ± 0.61	41.84 ± 2.44
C18:3n3	0.77 ± 0.07	0.62 ± 0.01	0.65 ± 0.02	1.03 ± 0.50
SFA	55.46 ± 0.05	45.68 ± 0.16	70.72 ± 0.29	45.88 ± 0.91
MUFA	13.68 ± 0.62	11.91 ± 0.04	7.98 ± 0.56	10.54 ± 2.19
PUFA	26.24 ± 0.33	41.51 ± 0.19	16.27 ± 0.52	42.91 ± 2.96
UFA	39.92 ± 0.95	53.42 ± 0.15	24.25 ± 0.04	53.45 ± 5.15
omega-6	25.47 ± 0.63	40.89 ± 0.18	15.55 ± 0.61	41.88 ± 2.46
omega-3	0.77 ± 0.07	0.62 ± 0.01	0.72 ± 0.09	1.03 ± 0.50
(b)				
Test		Statistic	<i>p</i> -value	
Mann–Whitney U test (coffee vs. by-products)		U = 181	0.62	
Kruskal–Wallis test (GC, RC, SS and SG)		H = 0.84, df = 3	0.84	
(c)				
Test		Statistic	<i>p</i> -value	
ANOVA (GC, RC, SS and SG)		F value = 0.16, df = 3	0.83	
Student's <i>t</i> -test (coffee vs. coffee by-products)		<i>t</i> = 0.23, df = 21.5	0.82	

C16:0—Palmitic acid; C18:0—Stearic acid; C20:0—Arachidic acid; C22:0—Behenic acid; C23:0—Tricosanoic acid; C24:0—Lignoceric acid; C18:1n9 *cis*—Elaidic acid; C20:1n9—*cis*-11-Eicosenoic acid; C18:2n6 *cis*—Linoleic acid; C18:3n3— α -Linolenic acid; SFA—Saturated Fatty Acids; MUFA—Monounsaturated Fatty Acids; PUFA—Polyunsaturated Fatty Acids; UFA—Unsaturated Fatty Acids; omega-6—Omega-6 fatty acids; omega-3—Omega-3 fatty acids. Values are means ± standard deviations of three (*n* = 3) measurements.

Palmitic acid (C16:0) was the main saturated fatty acid (SFA) in GC, RC, and SG, with quantities of 40.3%, 34.8%, and 34.4%, respectively. SS contained 19.3% palmitic acid and was a better source of behenic acid (C22:0), with a level of 20.7%. In comparison, RC, SG, and GC contained lower amounts of behenic acid, namely 0.4%, 0.6%, and 0.8%, respectively. Additionally, SS was a better source of arachidic acid (C20:0), lignoceric acid (C24:0), and tricosylic acid (C23:0), with levels of 18.3%, 5.0%, and 0.5%, respectively, than GC, RC, and SG (Table 2a). However, arachidic acid, lignoceric acid, and tricosylic acid were not detected or were present in low amounts in coffee and coffee by-products (Table S1, SM). With the exception of SS, which contained 8.9 to 15.4% behenic acid (Table S1, SM), the other three samples, GC, RC, and SG, showed lower levels of 0.5 to 0.7%, 0.2 to 0.7%, and 0.6 to 2.8%, respectively, consistent with the results of this study: 0.8% (GC), 0.6% (SG), and 0.4% (RC). Lignoceric acid was found in the studies of Bobkova et al. [59] and Vu et al. [66] for GC, RC, and SG, with levels of 0.2% and 0.3%, and 1.0 to 4.0%, respectively. These results were similar to those determined in this work: 0.2% (RC), 0.4% (GC), and

0.3% (SG). Somewhat higher levels of lignoceric acid, 1.7% and 2.3%, were reported by Senila et al. [64] for Robusta and Arabica RC varieties, respectively. Arachidic acid was found in higher quantities, ranging from 0.1 to 13.5% (SS), 0.94 to 3.4% (RC), 1.5 to 5.6% (SG), and 2.0 to 2.9% (GC) (Table 2a). The determined quantities were close to reported values (Table S1, SM) and increased in the following order: 2.6% (RC), 2.7% (SG), 3.6% (GC), and 18.3% (SS). In addition to palmitic acid, SS, compared with GC, RC, and SG, also contained a lower level of stearic acid (C18:0) of 5.2%, compared with 7.4%, 7.7%, and 9.4%, respectively. The stearic acid values were within the range reported by other authors (Table S1, SM), which spans 0.3 to 6.4% (SS), 5.6 to 9.0% (RC), 7.0 to 10.9% (SG), and 7.0 to 26.1% (GC). Oleic acid (C18:1-9cis) was the major monounsaturated fatty acid (MUFA) found in the following increasing order (Table 2a): SS (7.5%), SG (10.2%), RC (11.5%), and GC (12.7%). Other authors (Table S1, SM) have reported values of 0.2–8.3% (SS), 6.0–11.7% (RC), 7.2–13.3% (GC), and 8.0–12.7% (SG). Gondoic acid (C20:1n9), the second MUFA, was found in trace amounts (0.2–0.5%, depending on the sample) and has not been reported by other authors. In this study, GC contained 0.5%, followed by 0.4% (RC), 0.3% (SG), and 0.2% (SS). Linoleic acid (C18:2-9,12cis), the predominant polyunsaturated fatty acid (PUFA) (Table 2a), was the second most abundant fatty acid in GC, RC, and SG. The highest levels were found in RC (40.9%) and its by-product SG (41.8%), followed by lower amounts in GC (25.3%) and SS (15.6%). Other studies (Table S1, SM) have also reported high levels of linoleic acid: 1.3–31.5% in SS, 30.2–60.6% in RC, 39.7–46.5% in SG, and 43.4–51.6% in GC (with one exceptionally high value of 96.2%). Linolenic acid (C18:3-9,12,15cis), the second PUFA, was present in lower amounts, ranging from 0.6% to 1.0%, depending on the type of coffee and coffee by-product. Apart from the work of Senila et al. [64], which reported levels of 0.42% and 0.51% for Robusta and Arabica RC, this fatty acid has not been reported for coffee and coffee by-products. Due to the high individual SFA content in SS, the total fatty acid level is also high, contributing 70.7% (Table 2a). The total SFA content in GC was 55.5%, while in RC and SG it was similar, at 45.7% and 45.9%, respectively, as is the case for individual SFA. Although the data presented here agree with those reported (Table S1, SM), these findings indicate that a high proportion of long-chain SFA could serve as a potential indicator of sensory quality in coffee and coffee by-products, as they significantly affect the flavour profile. In comparison with SFA distribution, the total unsaturated fatty acids (UFA) were distributed among MUFA and PUFA (Table 2a), with oleic acid and linoleic acid as the main representatives. UFA content was lowest in SS (24.3%) compared to other samples, which contributed 39.9% (GC), 53.4% (RC), and 53.5% (SG). As linoleic acid was the main representative of PUFAs, its levels significantly influenced the total PUFA content, which was 16.3%, 26.2%, 41.5%, and 42.9% for SS, GC, RC, and SG, respectively, as well as the omega-6 content (Table 2a). Due to its high levels and omega-6 nature, the presence of linoleic acid in coffee and coffee by-products could be beneficial for health because of its role in prostaglandin biosynthesis and its contribution to systemic cell regeneration processes. In addition, this acid may have a positive effect on high-density cholesterol, reducing the risk of cardiovascular diseases. Therefore, based on the distribution of SFA, MUFA, and PUFA in coffees and coffee by-products, their nutritional indices—UFA/SFA (0.34–1.17), MUFA/SFA (0.11–0.26), PUFA/SFA (0.23–0.94), PUFA/MUFA (1.92–3.49), and omega-3/omega-6 (0.02–0.05), showed notable variability and reflect the fatty acid profiles of coffee and its by-products.

3.4. Influence of MAE Parameters on Bioactive Compound Content in Coffee and Coffee By-Product

Due to the lack of data on the MAE of coffee and coffee by-products, regardless of their subgroups, i.e., GC, RC, SS, and SG, the results obtained in this study were compared with recent literature reports, as described in the following paragraph. In addition, quantities

of bioactive compounds have been reported in review articles [3–6,9,15,17,24], mainly for SG, followed by SS, whereas for GC and RC there is limited literature evidence. These reviews reported that levels of bioactive compounds differ due to the extraction method and conditions, such as temperature, time, pressure, ultrasound intensity, coffee and coffee by-product varieties, solvent type, and notable differences in the expression of the results. As various investigations have shown, 50% and 70% (*v/v*) ethanol are the most commonly used solvents, so these were also applied in this study. In addition, time (5 and 15 min) and temperature (50 and 70 °C) were included to assess their suitability for further extraction of bioactive compounds from coffee and coffee by-products. The times and temperatures used were based on the work reported by Dobroslavić et al. [70] for MAE of laurel. The profiles and quantities of bioactive compounds in coffee and its by-products after MAE were analysed by UPLC-MS². The results showed the presence of caffeine and trigonelline as alkaloids; caffeoylquinic acids, including 3-caffeoylquinic acid (neochlorogenic acid), 5-caffeoylquinic acid (chlorogenic acid), and 4,5-dicaffeoylquinic acid; 3-feruloylquinic acid and 3-*p*-coumaroylquinic acid as derivatives of feruloylquinic acid and *p*-coumaroylquinic acid; and *p*-coumaric acid (Table 3).

As shown by the PCA of phenolic compounds PC1, PC2, and PC3 explained 68%, 24%, and 3% of the variance (Appendix A, Table A1, respectively, with plots showing significant differences ($p < 0.001$) among coffee and coffee by-product samples, i.e., GC, RC, SS, and SC (Figure 3).

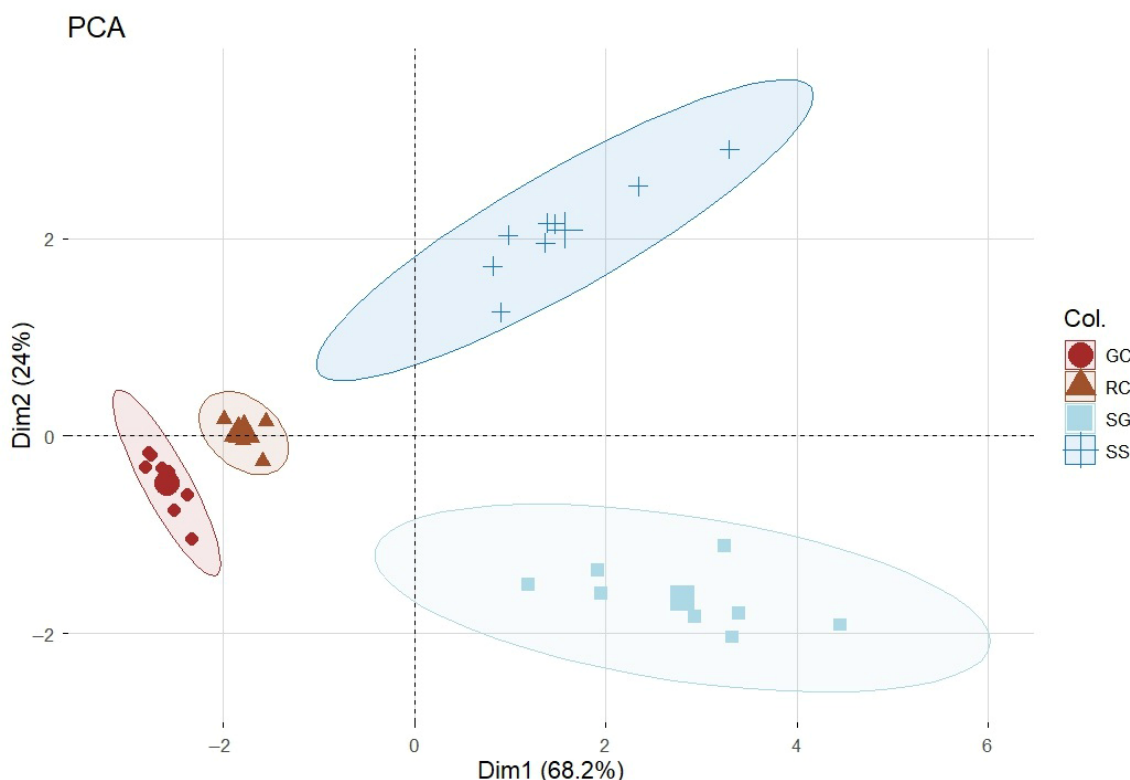


Figure 3. PCA plot of phenolic compounds in coffee (GC and RC) and coffee by-products (SS and SG) obtained by microwave-assisted extraction (MAE). The plot shows the first two principal components from the analysis, explaining 92% of the variation. Measurements are grouped by 95% confidence interval ellipses around the centroids of each group. Groups (GC, RC, SG, and SS) are significantly different ($p < 0.05$).

Table 3. Bioactive compounds in coffee and coffee by-products obtained by MAE using 50% and 70% (*v/v*) ethanol at temperatures of 50 °C and 70 °C for 5 and 15 min.

Identified Compound	Extraction Parameter							
	50 °C				70 °C			
	50% (v/v)		70% (v/v)		50% (v/v)		70% (v/v)	
	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min
	γ (Compound)/(mg/L)							
Green coffee								
caffeine	14.41 ± 0.57	12.37 ± 0.59	20.93 ± 1.53	17.49 ± 1.00	12.54 ± 0.68	9.64 ± 1.23	12.40 ± 0.77	13.95 ± 1.85
trigonelline	0.07 ± 0.04	0.08 ± 0.04	0.08 ± 0.03	0.07 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.05 ± 0.04	0.06 ± 0.01
4,5-dicaffeoylquinic acid	0.29 ± 0.06	0.30 ± 0.07	0.24 ± 0.07	0.24 ± 0.03	0.30 ± 0.08	nd	nd	0.11 ± 0.04
3-feruloylquinic acid	6.38 ± 0.36	6.18 ± 0.19	5.10 ± 0.11	5.27 ± 0.54	6.42 ± 1.08	1.58 ± 0.15	1.61 ± 0.28	2.84 ± 0.09
chlorogenic acid	12.45 ± 0.56	13.21 ± 0.35	10.71 ± 1.03	12.67 ± 0.69	13.47 ± 1.47	3.07 ± 0.45	4.51 ± 0.47	5.74 ± 0.23
neochlorogenic acid	3.71 ± 0.38	3.72 ± 0.78	3.04 ± 0.41	3.19 ± 0.28	3.79 ± 0.85	1.12 ± 0.07	1.23 ± 0.11	1.52 ± 0.25
3- <i>p</i> -coumaroylquinic acid	1.59 ± 0.13	1.75 ± 0.13	2.41 ± 0.17	2.52 ± 0.43	1.65 ± 0.24	0.43 ± 0.18	0.97 ± 0.09	1.16 ± 0.10
<i>p</i> -coumaric acid	0.48 ± 0.10	0.42 ± 0.06	1.11 ± 0.07	0.74 ± 0.20	0.68 ± 0.06	1.61 ± 0.18	1.20 ± 0.14	0.56 ± 0.10
Total sum	39.38 ± 1.15	38.03 ± 0.80	43.62 ± 2.20	42.19 ± 1.18	39.91 ± 1.69	17.52 ± 0.93	21.97 ± 0.96	25.84 ± 1.80
Roasted coffee								
caffeine	213.87 ± 9.14	230.62 ± 8.82	219.70 ± 3.84	209.47 ± 6.83	274.12 ± 7.65	245.47 ± 5.92	234.13 ± 6.07	225.96 ± 9.11
trigonelline	0.44 ± 0.11	0.53 ± 0.11	0.54 ± 0.17	0.55 ± 0.10	0.54 ± 0.14	0.53 ± 0.13	0.56 ± 0.10	0.51 ± 0.04
4,5-dicaffeoylquinic acid	1.47 ± 0.21	1.51 ± 0.07	0.86 ± 0.10	1.70 ± 0.07	2.05 ± 0.17	1.72 ± 0.12	0.74 ± 0.11	0.78 ± 0.20
3-feruloylquinic acid	2.18 ± 0.15	2.19 ± 0.12	2.36 ± 0.18	2.66 ± 0.04	3.74 ± 0.28	3.65 ± 0.23	3.12 ± 0.16	2.74 ± 0.14
chlorogenic acid	56.10 ± 2.62	54.69 ± 1.28	57.55 ± 2.87	57.28 ± 2.10	82.92 ± 6.16	71.35 ± 2.14	55.63 ± 2.71	62.14 ± 1.90
neochlorogenic acid	5.70 ± 0.60	5.36 ± 1.04	5.74 ± 0.19	6.20 ± 0.29	9.41 ± 1.58	4.40 ± 0.29	6.47 ± 0.56	6.72 ± 0.43
3- <i>p</i> -coumaroylquinic acid	0.87 ± 0.08	1.03 ± 0.09	1.17 ± 0.07	1.14 ± 0.06	1.33 ± 0.24	1.15 ± 0.16	1.04 ± 0.08	0.97 ± 0.12
<i>p</i> -coumaric acid	0.96 ± 0.14	0.95 ± 0.26	1.09 ± 0.10	0.65 ± 0.24	1.04 ± 0.17	0.98 ± 0.18	0.96 ± 0.11	1.46 ± 0.06
Total sum	281.59 ± 13.05	297.07 ± 9.24	289.01 ± 5.80	279.65 ± 8.89	375.15 ± 7.47	329.25 ± 3.89	302.65 ± 5.06	301.28 ± 6.70

Table 3. Cont.

Identified Compound	Extraction Parameter							
	50 °C				70 °C			
	50% (v/v)		70% (v/v)		50% (v/v)		70% (v/v)	
	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min
γ (Compound)/(mg/L)								
Silver skin								
caffeine	345.54 ± 9.36	293.79 ± 5.04	396.39 ± 13.83	561.14 ± 12.06	430.27 ± 12.23	324.98 ± 10.50	503.56 ± 13.78	425.74 ± 12.54
trigonelline	1.43 ± 0.13	1.26 ± 0.11	1.44 ± 0.12	1.88 ± 0.21	1.56 ± 0.16	1.39 ± 0.12	1.78 ± 0.13	1.53 ± 0.17
4,5-dicaffeoylquinic acid	3.17 ± 0.13	2.75 ± 0.11	2.76 ± 0.10	6.37 ± 0.14	4.28 ± 0.29	4.20 ± 0.44	5.25 ± 0.18	4.12 ± 0.21
3-feruloylquinic acid	69.67 ± 2.17	80.70 ± 5.71	82.94 ± 7.00	110.92 ± 5.62	89.49 ± 5.41	88.02 ± 5.62	97.73 ± 6.45	86.18 ± 3.90
chlorogenic acid	237.66 ± 7.02	252.48 ± 6.84	266.56 ± 9.73	344.12 ± 7.39	279.78 ± 5.67	283.47 ± 7.61	316.85 ± 10.95	286.74 ± 8.67
neochlorogenic acid	52.83 ± 2.25	49.02 ± 2.37	57.18 ± 2.50	85.62 ± 5.86	59.90 ± 6.12	61.68 ± 6.84	68.58 ± 7.13	53.92 ± 2.51
3- <i>p</i> -coumaroylquinic acid	11.44 ± 1.45	8.88 ± 0.59	8.70 ± 0.84	21.37 ± 1.97	10.09 ± 0.63	14.61 ± 1.92	16.66 ± 1.30	13.74 ± 0.98
<i>p</i> -poumaric acid	0.89 ± 0.08	1.57 ± 0.25	1.00 ± 0.10	1.39 ± 0.10	1.07 ± 0.15	0.93 ± 0.08	1.19 ± 0.12	1.04 ± 0.25
Total sum	722.63 ± 20.25	690.45 ± 12.46	816.97 ± 7.39	1132.81 ± 5.69	876.44 ± 6.41	779.28 ± 11.81	1011.80 ± 14.17	873.01 ± 4.28
Spent ground								
caffeine	125.79 ± 6.05	147.30 ± 7.29	150.43 ± 6.82	198.14 ± 10.39	162.89 ± 7.80	170.80 ± 6.16	170.10 ± 8.44	228.93 ± 7.32
trigonelline	2.45 ± 0.15	2.67 ± 0.15	2.80 ± 0.17	2.94 ± 0.18	2.68 ± 0.19	2.75 ± 0.18	2.80 ± 0.33	3.05 ± 0.21
4,5-diCQA	7.61 ± 0.59	13.32 ± 1.64	7.98 ± 0.39	10.40 ± 1.53	17.64 ± 2.70	20.28 ± 3.81	14.42 ± 1.85	27.32 ± 1.74
3-feruloylquinic acid	4.85 ± 0.40	6.37 ± 0.18	6.94 ± 0.37	8.02 ± 0.23	8.50 ± 1.94	9.52 ± 1.66	9.88 ± 0.40	11.24 ± 0.56
chlorogenic acid	289.59 ± 9.42	351.69 ± 8.71	398.54 ± 12.07	448.77 ± 8.82	454.08 ± 14.14	435.18 ± 10.72	524.88 ± 10.34	580.29 ± 11.08
neochlorogenic acid	39.53 ± 2.68	49.45 ± 6.38	50.38 ± 4.66	61.47 ± 3.79	62.47 ± 7.67	68.77 ± 6.05	70.60 ± 7.35	81.67 ± 6.12
3- <i>p</i> -coumaroylquinic acid	8.30 ± 1.12	10.18 ± 0.99	10.98 ± 1.25	23.78 ± 1.28	12.74 ± 1.46	13.26 ± 2.27	14.85 ± 1.10	16.69 ± 1.24
<i>p</i> -coumaric acid	2.15 ± 0.65	2.02 ± 0.16	2.06 ± 0.20	2.13 ± 0.14	2.27 ± 0.20	2.51 ± 0.46	2.56 ± 0.12	2.15 ± 0.17
Total sum	480.27 ± 2.32	583.00 ± 24.04	630.11 ± 15.63	755.65 ± 18.13	723.27 ± 4.61	723.07 ± 18.44	810.09 ± 14.40	951.34 ± 15.27

Values are means ± standard deviations of three ($n = 3$) measurements; nd—not detected.

In GC, caffeine is the predominant compound, ranging from 9.6 to 20.9 mg/L, followed by chlorogenic acid (3.1 to 13.5 mg/L), 3-feruloylquinic acid (0.1 to 6.4 mg/L), neochlorogenic acid (1.1 to 3.8 mg/L), *p*-coumaroylquinic acid (0.4 to 2.5 mg/L), *p*-coumaric acid (0.4 to 1.6 mg/L), 4,5-dicaffeoylquinic acid (0.1 to 0.3 mg/L), and trigonelline (0.05 to 0.08 mg/L). Baskaran and Radhakrishnan [71] also reported predominant amounts of caffeine (1.17 mg/mL) and chlorogenic acid (8.68 mg/mL) in GC, as did Canzi et al. [37], with levels of 1.01 and 0.98 g/100 g dw (caffeine) and 2.97 and 2.79 g/100 g dw (chlorogenic acid), depending on pollination (with and without). Pakosz et al. [72] found 1.19 g/100 g and 1.28 g/100 g dw of caffeine in Robusta and Arabica GC, respectively, whereas for RC they reported values of 0.46 g/100 g dw and 0.50 g/100 g dw, respectively. Awward et al. [73] showed caffeine levels of 166.72 mg/L in GC and 189.85 to 203.63 mg/L in RC, and chlorogenic acid levels of 543.23 mg/L in GC and 90.53 to 270.99 mg/L in RC. Liao et al. [74] reported 76.3 mg/g dw of chlorogenic acid in GC, 20.3 mg/g dw in light, and 6.4 mg/g dw in medium and dark RC. Caffeine and chlorogenic acid also predominate in the RC (Table 3); unlike previous studies, their concentrations are much higher than in GC. Caffeine content ranged from 209.5 to 274.1 mg/L, and chlorogenic acid from 54.7 to 82.9 mg/L, depending on MAE conditions (temperature and time) and the solvent used. The other compounds in RC are present in the following decreasing order: neochlorogenic acid (4.4 to 9.4 mg/L), 3-feruloylquinic acid (2.2 to 3.7 mg/L), 4,5-dicaffeoylquinic acid (0.7 to 2.1 mg/L), and trigonelline (0.44 to 0.56 mg/L). The values for *p*-coumaric acid (0.7 to 1.5 mg/L) were similar to those in GC, whereas RC contained lower levels of 3-*p*-coumaroylquinic acid (0.9 to 1.3 mg/L). Among the identified compounds in SS, caffeine also prevailed, ranging from 293.8 to 561.1 mg/L (equivalent to 10.8 to 18.7 mg/g of sample), depending on the MAE conditions. These data indicate that the roasting process significantly increases caffeine levels from green to roasted beans and also results in high quantities in SS. The highest caffeine yield was found in SG, at 125.8 to 228.9 mg/L (equivalent to 4.2 to 6.6 mg/g of sample), indicating that valuable amounts remain after brewing. This could be attributed to the short brewing time (approximately 5 to 10 min) and the high coffee-to-water ratio typical of the traditional preparation method used in this work. As a result, a substantial quantity of caffeine remained in the SG. In comparison, Iriundo-DeHonda et al. [43] reported caffeine contents of 24 and 53.3 mg/g in Arabica and Robusta SS samples prepared by stirring in water. Zengin et al. [75] also confirmed high caffeine levels in SG (32.84 to 54.44 mg/g) and SS (27.37 to 41.88 mg/g), depending on the solvents used in the ultrasonic bath treatment. Coffee by-products also contained a high amount of chlorogenic acid (Table 3), i.e., 289.6 to 580.3 mg/L (equivalent to 9.7 to 19.3 mg/g of sample) for SG and 237.7 to 344.1 mg/L (equivalent to 7.9 to 11.5 mg/g of sample) for SS, depending on MAE conditions. Compared with GC and RC, both by-products were better sources of neochlorogenic acid, 3-feruloylquinic acid, 4,5-dicaffeoylquinic acid, and trigonelline. In SS, these compounds were present as follows: 3-feruloylquinic acid (69.7–110.9 mg/L), neochlorogenic acid (52.8–85.6 mg/L), 4,5-dicaffeoylquinic acid (2.8–5.3 mg/L), and trigonelline (1.3–1.9 mg/L). SG had similar levels of neochlorogenic acid (39.5 to 81.7 mg/L) as SS, but contained lower amounts of 3-feruloylquinic acid (4.9 to 11.2 mg/L) and higher amounts of 4,5-dicaffeoylquinic acid (7.6 to 27.3 mg/L) and trigonelline (2.5 to 3.1 mg/L). SG also showed higher levels of *p*-coumaric acid (2.1 to 2.6 mg/L) than GC, RC, and SS (0.9 to 1.6 mg/L). Both by-products were better sources of 3-*p*-coumaroylquinic acid, found in similar quantities of 8.9 to 21.4 mg/L (SS) and 8.3 to 23.8 mg/L (SG). For comparison, Zengin et al. [75] reported similar quantities of chlorogenic acid, ranging from 7.61 to 14.10 mg/g and 4.26 to 8.91 mg/g in SG and SS, respectively, depending on the solvents used, as in this study. Several other research [5,45] also identified chlorogenic acid as the major phenolic compound in SG and SS. Rebollo-Hernanz et al. [5] reported amounts of 0.67 to 0.91 mg/g

for raw and ground silver skin samples, and Machado et al. [44] reported 52.53 mg/100 g in hydroethanolic solvent. Wen et al. [76] showed caffeine and chlorogenic acid quantities of 4.29 µg/mL and 2.81 µg/mL, respectively, at 38 W/cm² ultrasound intensity in 80% methanol. Anandre et al. [77] reported caffeine values of 194.1 to 391.9 mg/100 g dw, followed by chlorogenic acid (167.3 to 385.3 mg/100 g dw), neochlorogenic acid (8.3 to 104.1 mg/100 g dw), and 4,5-dicaffeoylquinic acid (3.6 to 11.0 mg/100 g dw), depending on coffee origin. Martínez-Inda et al. [78] reported chlorogenic acid contents of 8.12 to 61.04 µg/g dw, depending on ethanol fractions (25 to 100%). As shown, the results presented in this study and other reports differ due to several previously mentioned factors, including extraction method and conditions, temperature, time, pressure, coffee and coffee by-product varieties, solvent type, and so on.

Although PCA indicated that bioactive compounds were unevenly distributed among coffee and its by-product groups (GC, RC, SS, and SC) (Figure 3), no significant differences in compound quantities were observed with respect to the applied MAE parameters, including temperature (50 and 70 °C), time (5 and 15 min), and solvent. The PCA biplot in Figure S1a–c (SM) provides a visual overview of the multivariate variation in compounds across extraction conditions.

In addition to PCA, a non-parametric statistical analysis was performed on the score of the first principal component (Dim.1/PC1), which explained 68.2% of the total variance and thus captured the dominant pattern of chemical differences among samples. A highly significant ($p < 0.05$) separation between the two main categories, coffee and coffee by-products, as well as the four subgroups (GC, RC, SS, SG), was evident (Appendix A, Table A2), confirming that the primary PCA axis reflects strong compositional differences inherent to the origin of the samples. In contrast, extraction parameters (temperature, ethanol volume fractions, and extraction time) did not significantly affect Dim.1 (all $p > 0.3$), indicating that these processing factors contributed minimally to the major variance structure compared to the intrinsic chemical variability between sample groups. Although PCA indicated that temperature, time, and ethanol volume fractions did not contribute strongly to the main separation pattern in the scores plot, the linear mixed-effects model revealed that temperature and ethanol fractions significantly affected the Dim.1 values (Appendix A, Table A3). During model inspection, the factor time did not show a significant main effect or any substantial interaction influence. Furthermore, including time increased model complexity and reduced interpretability without improving explanatory power. Therefore, time was removed from the model in accordance with standard model-reduction practices to obtain a more stable final model. The refined model tested the interactive effects of group, temperature, and ethanol on scores while retaining the same random-effects structure. This discrepancy between PCA and mixed modelling was expected and reflects their different analytical principles. PCA is an unsupervised method that identifies the directions of greatest overall variance in the multivariate chemical dataset. In this study, the largest source of variation was the intrinsic chemical difference between the two sample matrices (coffee vs. by-products), which dominated the PCA structure and thus masked smaller but systematic effects of extraction parameters. The mixed-effects model, on the other hand, directly evaluates how each experimental factor contributes to changes in Dim.1 while statistically controlling for group-level structure (group and subgroups). Within this framework, both ethanol volume fractions and temperature showed significant positive effects, indicating that higher ethanol levels and elevated temperatures consistently increased the extraction of phenolic compounds, including chlorogenic acids, caffeoylquinic acids, and related hydroxycinnamate derivatives associated with positive loadings on Dim.1 scores. These molecules possess intermediate polarity: they contain both hydrophilic carboxylic and hydroxyl groups, as well as moderately hydrophobic aromatic rings. Thus, increasing

the ethanol volume fraction enhances its affinity for these mid-polarity phenolics, thereby increasing their solubility. Temperature showed a similar positive effect, reflecting its well-established role in accelerating diffusion, breaking weak intermolecular interactions within cell walls, and increasing solute solubility. At elevated temperatures such as 70 °C, phenolic acids more readily desorb from polysaccharide complexes, and the viscosity of the solvent decreases, allowing faster mass transfer. Therefore, when combined with 70% ethanol, the synergistic effect of both parameters achieved efficient extraction of mid-polarity bioactive compounds. Considering the results of the statistical analysis, where PCA and mixed modelling provide complementary perspectives, PCA highlights major global patterns, while the mixed model identifies meaningful factor-specific trends that remain hidden in unsupervised multivariate decomposition. It can be concluded that higher ethanol volume fractions (70%, *v/v*) and a temperature of 70 °C were the best MAE parameters for further bioactive compound recovery, regardless of the applied times.

Beyond the aforementioned statistical analysis, the effect size analysis (partial η^2) revealed that extraction temperature was the dominant factor affecting the concentration of all quantified compounds across coffee and by-product samples, while ethanol volume fraction and extraction time exhibited moderate to large effects, primarily through their interactions with temperature (Table S2, SM).

4. Conclusions

The proximate chemical composition, fatty acid profiles and quantities, as well as bioactive compound levels, indicate that coffee and its by-products are important sources of nutrients and bioactive compounds. Although both by-products have low protein (4.4% and 8.8%) and sugar (0.6% and 1.6%) contents, each exhibited a high level of total fibre (20.7–27.8%). They contain high amounts of saturated fatty acids, with palmitic acid being the most abundant in SG, RC, and GC (34.4–40.3%), while SS is a notably better source of behenic acid (20.7%). The unsaturated fraction of coffee and its by-products is mainly composed of oleic acid (7.5–12.7%) and linoleic acid (15.6–41.8%). FTIR spectroscopy analysis of oil from coffee and its residues confirms their triglyceride structure, showing the presence of saturated fatty acids and *cis* conformations of the double bonds in unsaturated fatty acids. Furthermore, MAE data indicate that it is an effective method for isolating alkaloids and phenolic acids, with chlorogenic acid and caffeine particularly prevalent in SS and SG. Although PCA results showed no significant impact ($p > 0.05$) of individual variables (solvent, temperature and time) on compound levels, the mixed model confirmed that solvent and temperature had a considerable impact ($p < 0.001$) on their amounts. Based on this, higher ethanol volume fractions (70%, *v/v*) and a temperature of 70 °C can be considered the best MAE parameters for further bioactive compound extraction.

In addition to the findings of this study, certain limitations should be noted, including differences among coffee and coffee by-products due to variety (Arabica, Robusta, or their blends), geographical location, and specific processing conditions such as roasting degree (light, medium, or dark), brewing techniques (Turkish, French press, Aeropress, espresso, and simple infusion), and by-product collection methods. These differences may influence the amounts of target analytes and their applicability. Furthermore, the safety and long-term stability of the extracts obtained by MAE were not evaluated. Consequently, future research should focus on a comprehensive comparison of coffee and coffee by-products from diverse geographical locations and detailed characterisation of extracts to assess their suitability for food and nutraceutical use.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16041794/s1>, Table S1: Summary of fatty acid content in coffee and coffee by-products in the recently reported studies; Figure S1. PCA biplot of phenolic compounds in coffee and coffee by-products obtained by MAE. The plot shows the first two principal components from the analysis, explaining 92% of the variation. Measurements are grouped by ethanol volume fractions, with 95% confidence interval ellipses around the centroids of each group. (a). The groups (50% ethanol, 70% ethanol) are not significantly different ($p = 0.56$). (b). The groups (50 °C, 70 °C) are not significantly different ($p = 0.38$). (c). The groups (5 min, 15 min) are not significantly different ($p = 0.38$). Table S2. Effect size analysis (partial η^2) of bioactive compounds of coffee and coffee by-products.

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Abbreviations

The following abbreviations are used in this manuscript:

GC	Green coffee
RC	Roasted coffee
SS	Silver skin
SG	Spent grounds
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
UFA	Unsaturated fatty acid
MAE	Microwave-assisted extraction
FTIR	Fourier transform infrared spectroscopy
UPLC-MS ²	Ultra-Performance Liquid Chromatography–Mass Spectrometry

Appendix A

Table A1. Principal component analysis (PCA) of bioactive compounds in coffee and coffee by-products during MAE.

	PC1	PC2	PC3
Standard deviation	2.33	1.38	0.49
Proportion of Variance	0.682	0.24	0.03
Cumulative Proportion	0.682	0.92	0.95

Table A2. Results of non-parametric statistical analyses performed on the scores of the first principal component (Dim.1/PC1) of bioactive compounds in coffee and coffee by-products during MAE.

Test	Statistics	p-Value
Mann–Whitney U test	Group (coffee vs. coffee by-products)	U = 256
Kruskal–Wallis test	Subgroup (GC, RC, SS, SG)	H = 27.44, df = 3
Mann–Whitney U test	Temperature (50 vs. 70 °C)	U = 104
Mann–Whitney U test	Ethanol (50% vs. 70%)	U = 112
Mann–Whitney U test	Time (5 vs. 15 min)	U = 135

Significant values are highlighted in **bold**.**Table A3.** Fixed effects from the linear mixed-effects model predicting PCA Dim.1 scores of bioactive compounds during MAE.

Effect	Estimate	Std. Error	df	t-Value	p-Value
(Intercept)	1.218	1.012	16.78	1.203	0.246
GROUP (COFFEE)	−3.533	1.431	16.78	−2.468	0.025
TEMPERATURE (70 °C)	1.035	0.396	22.00	2.614	0.016
ETHANOL (70%)	1.142	0.396	22.00	2.883	0.009
GROUP × TEMPERATURE	−0.784	0.560	22.00	−1.399	0.176
GROUP × ETHANOL	−1.007	0.560	22.00	−1.798	0.086
TEMPERATURE × ETHANOL	−0.481	0.560	22.00	−0.858	0.400
GROUP × TEMPERATURE × ETHANOL	0.225	0.792	22.00	0.284	0.779

Significant values are highlighted in **bold**.

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