LWT - Food Science and Technology 198 (2024) 115974

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Exploitation of spent coffee ground (SCG) as a source of functional compounds and growth substrate for probiotic lactic acid bacteria

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ARTICLE INFO

Keywords:
Spent coffee ground
Antioxidants
Food waste
Functional food
Biotechnologies

ABSTRACT

Spent coffee ground (SCG) represent a food waste with functional potential, produced in high amount worldwide. SCG derive from coffee brewing and are mainly composed of insoluble matter and different efforts is still necessary to find innovative processes for their valorization. In this work, different methodologies including physical (cryogenic milling and microwave assisted extraction, MAE) and biological (optimized enzyme-assisted extraction, EAE) were exploited to solubilize compounds trapped within the abundant fiber network. MAE led to the highest concentration of soluble fiber and oligosaccharides thus solubilizing insoluble fiber. The total soluble matter increased almost 8 folds by using MAE and EAE in combination due to high insoluble fiber hydrolysis into monosaccharides (up to 17 g/100g), soluble melanoidins (up to 72 mg/g) and caffeic acid (up to 2.22 mg/g). The extract was also characterized for the highest antioxidant potential demonstrating the positive impact of combined process. The EAE promoted the release of nutrients in SCG extract which were used as a growth substate by the selected probiotic *Lactiplantibacillus plantarum* LP19. This work demonstrates how different technologies and their combination can be used to valorize SCG demonstrating the possibility to obtain novel SCG-derived functional ingredients and/or products.

1. Introduction

The food system is rapidly transitioning to the production of higher amounts of products to feed a growing population. As a drawback, high levels of food loss and waste are generated thus requiring a large-scale agro-food by-product management (García-Oliveira, Fraga-Corral, Pereira, Prieto, & Simal-Gandara, 2022). Coffee is a widely consumed and highly valued product worldwide (Tucker, 2017) which consumption is continuously increasing reaching around 10.1 billion kg in 2020, 1 billion more than in 2015 (International Coffee Organization, 2021). All steps of coffee processing generate a substantial amount of waste and only the coffee brewing produce around 6 million tons of spent coffee ground (SCG) annually (Getachew & Chun, 2017). Recent research efforts have focused on studying the composition of SCG for their potential reuse, as suggested by the principle of circular economy. Polysaccharides, oligosaccharides, lipids, aliphatic acids, amino acids, proteins, alkaloids (e.g. caffeine, trigonellin), phenols, minerals, lignin, melanoidins, and volatile compounds are valuable constituents of SCG (Campos-Vega, Loarca-Pina, Vergara-Castañeda, & Oomah, 2015; Mussatto, Carneiro, Silva, Roberto, & Teixeira, 2011; Pujol et al., 2013). SCG possess functional qualities, due to the high quantity of fibers, tannins, and phenols, which result in high antioxidant potential. SCG fibers are largely insoluble and mostly composed of cellulose and hemicellulose, with a large amount of lignin (Ballesteros, Teixeira, & Mussatto, 2014). Additionally, the SCG has a substantial amount of lipids and proteins with high biological value (Campos-Vega et al., 2015; Martinez-Saez et al., 2017). SCG has been proposed as bulk material for several uses including adsorbers, fillers, additives for polymers production, supplement in animal feed, and soil fertilizers. However, SCG is a source of valuable bioactive compounds. Diterpene alcohol esters and

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https://doi.org/10.1016/j.lwt.2024.115974
Received 11 October 2023; Received in revised form 26 February 2024; Accepted 15 March 2024
Available online 16 March 2024

phenolics represent the most prominent ones (Kovalcik, Obruca, & Marova, 2018) while condensed and hydrolysable tannins are additional active polyphenols found in SCG (Low, Rahman, & Jamaluddin, 2015). Melanoidins are end-products of the Maillard reaction with high molecular weight and variable composition, usually incorporating also SCG phenolics (Borrelli et al., 2003). Melanoidins are associated with different biological activities including prebiotic, antioxidant, and antimicrobial (Pérez-Burillo, Rajakaruna, Pastoriza, Paliy, & Rufián--Henares, 2020). Dietary melanoidins bypass digestive processes in the gastrointestinal tract becoming substrates for the production of short chain fatty acids (SCFAs) by gut bacteria and then modulating the microbiota (Pérez-Burillo et al., 2020; Rufián-Henares & Pastoriza, 2015; Wang, Qian, & Yao, 2011). Besides melanoidins, mannooligosaccharides (MOS) are defined prebiotic due to their ability to influence the gut microbiota by promoting the proliferation of particular advantageous species (Pérez-Burillo et al., 2019). Therefore, SCG derived ingredients should be used as such or utilized for the growth of microorganisms, such as probiotic lactic acid bacteria, considering the high concentration of fibers and melanoidins with prebiotic potential (Wongsiridetchai, Jonjaroen, Sawangwan, Charoenrat, & Chantorn, 2021). Polar or intermediately polar solvents are usually employed for the extraction of these compounds from food by products. Unfortunately, most of the coffee valuable molecules were extracted during brewing and the remaining in SCG are trapped within the abundant fiber network, therefore an extensive hydrolysis is required to extract and use them as a source of functional ingredients. Enzyme-assisted extraction (EAE) is very promising for achieving an efficient and cost-effective hydrolysis of plant matrices (Corrêa, Penha, Freitas-Silva, Luna, & Gottschalk, 2021; Ravindran, Jaiswal, Abu-Ghannam, & Jaiswal, 2017). Due to the high levels of lignin, cellulose, and hemicellulose, different enzymes including β-glucanases, cellulases, and hemicellulases can be used to solubilize SCG insoluble components. Among these, Viscozyme®L and Celluclast®1.5L are two enzymes successfully tested for the hydrolysis of food matrices and SCG (Guan & Yao, 2008; Hudeckova, Neureiter, Obruca, Frühauf, & Marova, 2018; Phirom-on & Apiraksakorn, 2021). Besides enzymes, physical methods can be used to hydrolyze SCG matrix and especially microwave treatment (Microwave Assisted Extraction, MAE) was very effective to extract oligosaccharides and the phenolic fraction (Passos & Coimbra, 2013; Pettinato, Casazza, & Perego, 2019; Solomakou et al., 2022). Central Composite Design (CCD) was used for SCG extraction of phenols (Coelho, Robalo, Boyadzhieva, & Stateva, 2021; Zhang, Yang, Wang, & Rupasinghe, 2021; Gigliobianco et al., 2020), and polysaccharides (Zhang et al., 2021) using different physical methods (ultrasounds and microwaves), solvents, and temperature combinations. Both for enzymatic and physical extraction methods the particles size is an important influencing factor for the extraction of phenolic compounds (Pinelo, Tress, Pedersen, Arnous, & Meyer, 2007).

The aim of this work is to investigate the effect of physical and/or enzymatic treatments on the increase of soluble components and functional properties of SCG. Moreover, a biotechnological protocol designed for turning SCG into a suitable substrate for probiotic microorganisms growth was set-up, aiming at exploiting the possibility to obtain novel SCG-derived functional ingredients.

2. Material and methods

2.1. Raw materials, media and chemicals

Spent coffee ground (SCG) was collected from the local cafeteria in the Impulse building (Wageningen, Netherlands) deriving from coffees prepared with the IB7 coffee machine (Iberital, Spain) and organic coffee grains beans (Peru medium roasting, Peeze, Netherland). De Man, Rogosa, and Sharpe (MRS) agar, Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Plate Count Agar (PCA), and Violet Red Bile Glucose Agar (VRBGA) were provided by Oxoid (Basingstoke, Hampshire, United Kingdom). Viscozyme®L (100 FBG/g), consisting mainly in endo-β-glucanase that hydrolyzes (1,3)- or (1,4)-linkages in beta-D-glucans, and Celluclast®1.5L (700 EGU/g), providing cellulase that hydrolyzes (1,4)- β-D-glucosidic linkages in cellulose and other β-D-glucans, were provided by Novozymes (Bagsværd, Denmark). HPLC standards were obtained in analytical standard quality (purity ≥97%) from Sigma-Aldrich (St. Louis, USA) while solvents from VWR International (Radnor, USA).

2.2. Spent coffee grounds stabilization and characterization

SCG was dried in the optimal range temperature and time (Martinez-Saez et al., 2017) at 55 °C for 24 h in a Binder DF56 incubator (BINDER GmbH, Germany). SCG was analyzed before and after the stabilization treatment for its dry matter (d.m.) and the moisture content according to 44–15.02 method (AACC, 2010), and its microbiological characterization. In details, 10 g of SCG before and after drying were homogenized with 90 mL of sterile peptone water (1%, w/v) at 200 rpm for 3 min. Lactic acid bacteria were enumerated using MRS supplemented with cycloheximide (0.1 g/L), yeasts using MEA, moulds using PDA, total mesophilic aerobic bacteria using PCA, and enterobacteria using VRBGA.

2.2.1. Proximal composition of spent coffee grounds

Moisture was determined as previously mentioned, while ash content according to AACC method n. 08–01 (AACC, 2010). Protein content was evaluated by the Dumas method using a Flash EA 1112 NC analyser (Thermo Fisher Scientific Inc., Waltman, USA) and the conversion factor 6.25 (Massaya, Pereira, Mills-Lamptey, Benjamin, & Chuck, 2019). Fat content was determined after extraction by Soxhlet apparatus by using 5 g of freeze-dried SCG and 200 mL of petroleum ether. Soluble and insoluble fibers were determined according to AACC Method n. 32–07.01 (AACC, 2010) by using K-TDFR-200A kit (Megazyme, Ireland). The carbohydrates were determined by difference (FAO, 2003).

2.3. Set up of enzymatic bioprocess by using central composite design (CCD)

2.3.1. Enzymes

Viscozyme®L and Celluclast®1.5L were firstly tested according to the suggested dosages of 200–400 mL and 100–200 mL per ton of vegetable, the optimal pH range 3.3–5.5 and 4.0–6.0, and optimal temperature 40–50 and 50–60 °C, respectively (https://www.novozymes.com.cn/en/en/juice-fruit-vegetables/products/vegetables/viscozyme-l; https://www.novozymes.com.cn/en/en/juice-fruit-vegetables/products/vegetables/celluclast-1.5-l).

2.3.2. Optimization of the enzymatic treatment by using CCDs

A preliminary assay was performed evaluating time (2–14 h), temperature (from 25 to 45 °C), pH (from 4.65 to 5.65), and enzyme concentrations (from 0.4 to 4 μL of Viscozyme®L/g of SCG and from 0.2 to 2 μL of Celluclast®1.5L/g of SCG). A mixture of SCG and demineralized water (1:3 w/w) was put into 50-mL Greiner centrifuge tubes (Sigma, USA), which were covered and kept in a Binder DF56 incubator (BINDER GmbH, Germany), setting appropriate temperature and time. A SCG extract was also obtained by incubation of SCG with demineralized water in ratio 1:3 w/w for 1 h. The tubes were then centrifuged (4700×*g*, 4 °C, 10 min), and the supernatant (SCG extract) was filtered through 0.22 μm filters (Sigma Aldrich) and kept at −20 °C in the dark until analysis. For further optimization, 14 h was selected and the EAE was optimized by Non-Factorial Central Composite Design (CCD) of Statistica 10 (StatSoft Inc., USA including 26 runs with three central points. The effect of pH (5.15–5.95), temperature (45–55 °C), and Viscozyme®L (0–20 μL/g SCG) and Celluclast®1.5L (0–10 μL/g SCG) concentrations was evaluated. The critical values after data analysis

were the highest pH (5.95) and temperature (55 °C) tested. In contrast, higher values of enzymes than the ones tested were found, requiring the optimization of the model. Both Viscozyme®L and Celluclast®1.5L resulted in the most important variables. Therefore, higher concentrations of Viscozyme®L/g (20, 50, and 80 μL/g of SCG) and Celluclast®1.5L (10, 25, and 40 μL/g of SCG) in a full factorial design (3^2) were tested.

2.4. Set up of physical and combined physical-enzymatic treatments

2.4.1. Cryogenic grinding and particle size evaluation

SCG were finely grinded by using the cryo-miller 6875D Freezer/Mill® (Spex Sample Prep, United States). In details, 20 g of SCG were milled three times for 5 min with 10 cycles per second. The particle size distribution was analyzed using the Mastersizer 3000 (Malvern Instruments, Worcestershire UK) equipped with Aero S and the particle refractive index of 1.530 (Cervera-Mata, Lara, Fernández-Arteaga, Rufián-Henares, & Delgado, 2021). Values of D[3,2], D[4,3], D(10), D(50), and D(90) were obtained.

2.4.2. Microwave assisted extraction

The Monowave 400 (Anton Paar, Austria) was used mixing 4 g of SCG and 12 g of demineralized water in a glass vial G30 (Anton Paar, Austria). The process was performed as previously described by Rodrigues et al. (2020) with some modifications. In details the stirring settings were at 300 rpm and the maximum power of 850 W to reach 200 °C as fast as possible, then the temperature was maintained for 10 min and after decreased to 55 °C. All the samples were frozen at −20 °C before subsequent treatment or analysis.

2.4.3. Combination of mechanical and enzymatic treatments

The previously optimized EAE was applied to SCG after either cryogenic grinding (SCG-C) or MAE (SCG-M) thus obtaining SCG-CE and SCG-ME, respectively. The sample preparation is summarized in Supplementary Fig. 1.

2.5. Chemical characterization

2.5.1. Total soluble fraction, soluble and insoluble fiber

The quantification of the soluble fraction released after treatments was evaluated in SCG extracts obtained after centrifugation at 4700×*g* at 4 °C for 10 min according to 44–15.02 method (AACC, 2010). Soluble and insoluble fibers were determined according to AACC Method n. 32–07.01 by using K-TDFR-200A Megazyme kit (Megazyme, Ireland).

2.5.2. Sugars and oligosaccharides

The sugar and oligosaccharide composition was determined according to de Keijzer, Van den Broek, Ketelaar, and Van Lammeren (2012) by High Performance Anion Exchange Chromatography (HPAEC). The ICS 5000+ ion chromatography HPLC system equipped with a CarboPac PA-1 column (2 × 250 mm) in combination with a pulsed electrochemical detector in pulsed amperometric detection mode (Thermo Fischer Scientific) was used. A flow rate of 0.25 mL min^{-1} was used and the column was equilibrated with 21 mM NaOH at 30 °C. The following gradient was used: 0–20 min, 21 mM NaOH, 20–30 min, 21–100 mM NaOH, 30–90 min, 0–0.3 M sodium acetate in 100 mM NaOH, 90–91 min, 0.3–0.5 M sodium acetate in 100 mM NaOH, 91–96 min, 0.5 M sodium acetate in 100 mM NaOH, 96–97 min, 0.5 M sodium acetate in 100 mM NaOH to 21 mM NaOH, 97–112 min, 21 mM NaOH. The oligosaccharide concentration was calculated as galactobiose equivalent evaluating the total peak area of chromatograms from the 30th to the 100th minute. The LOD and LOQ values were calculated by using the calibration curve and standard deviation of the intercept approach (Supplementary Table 1).

2.5.3. Caffeic and chlorogenic acids

Caffeic and chlorogenic acids analysis was carried out with a Nexera UPLC system (Shimadzu Corporation, Kyoto, Japan) coupled with a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The UPLC unit consisted of a SIL-30AC autosampler, a LC-20ADXR solvent delivery module, DGU-20ASR degassing unit, a CTO-20AC column oven and a FCV-20AH2 valve unit. The samples (5 μL) were injected on a Kinetex Evo C18 column (2.6 μm, 2.1 × 100 mm, 100 Å, Phenomenex, Torrance, CA, USA) by using the setting previously described by Peña-Correa, Mogol, Fryganas, and Fogliano (2023). In details, the flow rate was set at 0.5 mL/min and the column temperature at 40 °C. The mobile phases consisted of 0.2% formic acid (solvent A), acetonitrile with 0.1% formic acid (solvent B) with the following elution profile (t in [min]/[%B]): (0.0/5), (0.5/5), (2.0/25), (5.0/50), (7.0/95), (8.5/95), (8.60/5) and (12.5/5). The voltage of the turbo ion-spray ionization was 4.0 kV. The temperature of electrospray ionization probe, desolvation line, and heat block were set at 300, 250, and 400 °C, respectively. The pressure of the collision-induced dissociation gas was 4 kPa whereas the flow rates of the drying gas, nebulizer gas, and heating gas were set at 10 mL/min, 3 mL/min, and 10 mL/min, respectively. The electrode voltage of Q1 pre bias, collision cell Q2, Q3 pre bias, parent and fragment ion m/z of the multiple reaction monitoring transitions were optimized using standard solutions of the target analytes (concentration 0.10–10 ppm) and the support software (Shimadzu Corporation, Kyoto, Japan). Dwell time and the time window for MS data acquisition, in both ionization modes, were also optimized for single reaction monitoring (SRM) (Supplementary Table 2) (Peña-Correa et al., 2023). The LOD and LOQ values were calculated as previously described (Supplementary Table 1).

2.5.4. Melanoidins

Melanoidin concentration was determined at 405 nm, using the extinction coefficient of 0.7 L/cm/g developed by Bekedam, Schols, Van Boekel, and Smit (2006) by using Cary 60 spectrophotometer (Agilent Technologies Inc.) and a glass fiber probe with a path length of 1 cm.

2.6. Antioxidant activity

The antioxidant activity of SCG extracts was evaluated by DPPH, ABTS, and ferric reducing ability of plasma (FRAP) assays. DPPH assay was performed as previously described by Brand-Williams, Cuvelier, and Berset (1995) preparing a 1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, USA) in methanol. Then, 20 μL of samples or Trolox (Sigma, USA) standard solutions (from 0 to 1 mM) were added to 780 μL of methanol-DPPH solution. The absorbance was recorded after 30 min of incubation at room temperature in dark.

ABTS assay was performed according to Re and colleagues (1999) measuring the absorbance at 734 nm of samples or Trolox standard solutions (from 0 to 1.5 mM) while FRAP assay was performed as described by Benzie and Strain (1996) preparing FRAP reagent freshly and measuring absorbance at 593 nm of samples or ferrous sulphate standard solutions (from 0 to 1 mM).

2.7. Choose of the probiotic strain and evaluation of lactic acid bacteria growth in SCG

SCG and SCG-E extracts were used to select commercial probiotic microorganisms as indicators for probiotic growth in SCG before and after physical and enzymatic treatments. The microorganisms used were *Bifidobacterium animalis* B501, *Limosilactobacillus fermentum* LF08, *Lactiplantibacillus plantarum* LP01, LP14, and LP19, *Lacticaseibacillus rhamnosus* ATCC 53103, *Lactobacillus acidophilus* LA02, *Lacticaseibacillus paracasei* LPC13, LPC00, and LPC2, and *Levilactobacillus brevis* LB17. All the strains were previously characterized for the probiotic activity and were kindly provided by Probiotical (Novara, Italy) and Proge Farm (Novara, Italy). Strains were regularly grown at 30 °C for 24 h in MRS

and collected by centrifugation at 4700×*g* at 4 °C for 10 min, washed twice with physiological salt solution, resuspended, and then inoculated to approximately 7 log10 cfu/mL. The growth capability assay was performed for 24 h by using SpectraMax M2 Multi-Mode Microplate reader (Molecular Devices, USA) set at 30 °C with readings every 60 min at 600 nm. When significant cell density increase during fermentation was detected, data were modelled according a modified Gompertz equation (Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990). *L. plantarum* LP19 was selected as the best probiotic bacteria in treated SCG due to the best growth parameters after 24 h of incubation. Therefore, the microbial suspension obtained as previously described was used to inoculate SCG, SCG-C, SCG-M, SCG-E, SCG-CE, and SCG-ME. Microbial growth and pH were monitored after incubation of 24 h at 30 °C in MRS agar as previously described.

2.8. Statistical analysis

All the samples were prepared in duplicates while the analyses were carried out in triplicate. Data were subjected to one-way ANOVA and pair-comparison of treatment means was achieved by Tukey's procedure at $P < 0.05$. Pearson correlation coefficient ($P < 0.05$) was calculated to discuss antioxidant analyses results. The CCD set up, CCD results evaluation, and all statistical analyses were performed using the statistical software Statistica 10 (StatSoft Inc., Tulsa, USA).

3. Results

3.1. Spent coffee ground stabilization and characterization

The thermal stabilization of SCG led to a significant decrease in the water content (from 62.03 ± 3.35 to 9.03 ± 1.36%). A significant decrease of microbial cell density was found in all the microbial groups considered. Moulds were not detected in both SCG samples, before and after heating treatment (Table 1). Considering the microbiological characterization results normalized on dry matter basis, a decrease of 2.42 log10 cfu/g in the total mesophilic bacteria was found after 24 h at 55 °C. Similarly, significant decreases were found in the cell densities of enterobacteria, yeasts, and LAB (-0.94 log10 cfu/g, - 2.09 log10 cfu/g, and - 1.19 log10 cfu/g, respectively) (Supplementary Table 3).

The high fibre content was mainly represented by the insoluble fraction (50.24 ± 2.27 g/100g), while the soluble fibres were 5.46 ± 0.36 g/100g. The total fat content was 14.99 ± 0.02 g/100g, while the protein fraction, ashes, and carbohydrates were 12.83 ± 0.15 g/100g, 1.05 ± 0.05 g/100g, and 6.40 ± 2.86 g/100g, respectively (Table 2).

3.2. Optimization of the enzymatic treatment

Viscozyme®L and Celluclast®1.5L were preliminary tested by evaluating the conditions proposed by the producer. Despite the increase in water extractable soluble matter after the treatment from 3.98 ± 0.11 to 5.27 ± 0.18% of SCG, the EAE required a further optimization considering only the treatment time of 14 h. Overall, increases in the supernatant soluble substances were obtained when highest enzyme concentrations, temperature and pH were used, and the highest concentration corresponded to 9.38% of SCG. Pareto chart was plotted to verify the effect of each variable in the responses (Supplementary Fig. 2).

The most important variable for the solubilization of SCG matter resulted in enzymes concentrations while pH and temperature were also significant variables. The R square of the model was 0.9937 and the mean square residual was 0.0256 thus confirming a strong correlation between predicted and observed values (Supplementary Fig. 2).

The CCD model showed the critical values of pH and temperature in the range tested corresponding to 4.91 and 55 °C but out of the range for the enzymes concentrations, thus requiring a further investigation. A full factorial CCD was performed evaluating concentration up to 80 μL of Viscozyme®L/g of SCG and 40 μL of Celluclast®1.5L/g of SCG. Therefore, the optimized model showed that the optimal Viscozyme®L and Celluclast®1.5L concentrations were 66.0 and 33.6 μL/g of SCG, respectively (Fig. 1).

Table 1
Microbiological characterization of spent coffee grounds before (Raw SCG) and after (Dried SCG) thermal stabilization process.

	Raw SCG (log10 cfu/g)	Dried SCG (log10 cfu/g)
Total aerobic mesophilic bacteria	4.76 ± 0.11[a]	2.72 ± 0.37[b]
Enterobacteriaceae	2.23 ± 0.18[a]	1.67 ± 0.26[b]
Yeasts	3.69 ± 0.42[a]	1.98 ± 0.23[b]
Moulds	<100 cfu/g	<100 cfu/g
Lactic acid bacteria	3.28 ± 0.34[a]	2.47 ± 0.19[b]

[a–b]Values in the same column with different superscript letters differ significantly ($p < 0.05$).

Table 2
Proximal composition of spent coffee grounds after thermal stabilization process.

	g/100g
Fat	14.99 ± 0.02
Protein	12.83 ± 0.15
Insoluble fiber	50.24 ± 2.27
Soluble fiber	5.46 ± 0.36
Carbohydrates	6.40 ± 2.86
Ash	1.05 ± 0.05
Moisture	9.03 ± 0.36

3.3. Cryo-milling and microwave assisted extraction

The granulometry of dried SCG was characterized by the following parameters: D[3,2] of 102.8 ± 2.6 μm, D[4,3] of 372.4 ± 7.2 μm, D(10) of 34.2 ± 0.5 μm, D(50) of 292.3 ± 4.9 μm, and D(90) of 638 ± 11.7 μm. The cryo-milling process led to a significant decrease in SCG particle size. Indeed, SCG-C was characterized by D[3,2] of 33.7 ± 0.6 μm, D [4,3] of 93.6 ± 3.3 μm, D(10) of 15.0 ± 0.2 μm, D(50) of 44.4 ± 0.9 μm, and D(90) of 243.1 ± 8.5 μm. Therefore, the highest reduction was

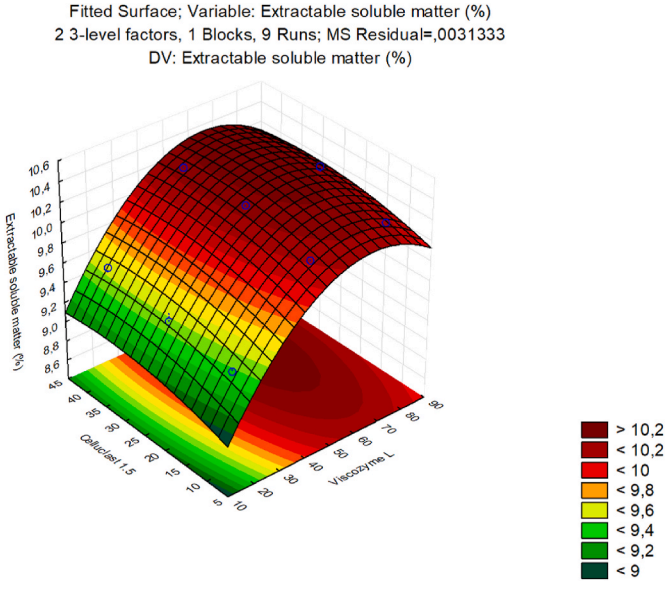

Fig. 1. Response surface plot for the optimization of enzymes concentrations in a full factorial design (3^2). The incubation time was 16 h, pH of 4.95 and temperature of 55 °C.

found in D(50), resulting in a particle size reduction of 85%.

The treatment by using Monowave 400 needed 145 s to reach the temperature of 200 °C with the maximum pressure of 30 bar. The total time for the treatment was approximately 27 min.

3.4. Chemical characterization

3.4.1. Soluble fraction, fiber, sugars and oligosaccharides

The soluble matter was evaluated in all the extracts ranging from 3.92 ± 0.24 to 33.53 ± 0.48 g/100g of SCG. The cryogenic milling led to the increase of extracted soluble matter of 40 % in SCG-C compared to SCG and of 30 % in SCG-CE compared to SCG-E (Fig. 2). The MAE showed the best results in terms of solubilization of SCG matrix reaching 20.31 ± 0.43 g/100g and 33.53 ± 0.48 g/100g of soluble matter in SCG-M and SCG-ME, respectively.

The composition in soluble and insoluble fibre was investigated in freeze dried samples showing the highest concentration of insoluble fibre and the lowest concentration of soluble fibre in SCG (Supplementary Fig. 3A). A general decrease in insoluble fibre and increase in soluble fibre was found after treatments except for the cryogenic milling. The EAE did not significantly decrease the insoluble fibre in SCG while a slight increase in oligosaccharides was detected (from 0.41 ± 0.03 to 1.22 ± 0.05 g/100g of SCG d.m.). The reduction of particle size and the subsequent EAE in SCG-CE decreased the insoluble fibre (47.26 ± 0.48 g/100g of SCG d.m.) and increased soluble fibre (7.65 ± 0.36 g/100g of SCG d.m.) with no statistical differences in oligosaccharides concentration when compared to SCG (Supplementary Fig. 3B). The lowest concentration of insoluble fibre was found in SCG-ME (30.67 ± 0.39 g/100g of SCG d.m.), thus confirming the capability of the combination of MAE and EAE treatments to solubilize fiber. However, the highest concentration in soluble fiber and oligosaccharides was detected in SCG-M (20.63 ± 3.26 g/100g of SCG d.m. And 3.89 ± 0.20 g/100g of SCG d.m., respectively) while significant decreases of 57% and 85% in soluble fiber and oligosaccharides, respectively, were found when the EAE was applied to SCG-M.

More than 17 g/100g of sugars was found in SCG-ME, which corresponded to the highest concentration among samples. Mannose, galactose, and glucose were the sugars detected at highest concentrations in SCG-ME (Fig. 3). Only 1.12 g/100g of sugar were found in SCG-M of which 0.67 g/100g was arabinose. As expected, the total sugar concentration in SCG and SCG-C was lower than 0.1 g/100g, while a significant effect of the cryogenic milling was found in SCG-CE corresponding to the increase of mannose, galactose, glucose, and arabinose from 25 to 75% compared to SCG-E. The oligosaccharides highest concentration was found in SCG-M (3.89 ± 0.20 g/100g) while the lowest in SCG (0.41 ± 0.03 g/100g). The optimized EAE increased oligosaccharides when applied directly to SCG and SCG-C (Supplementary Fig. 3B).

3.4.2. Caffeic and chlorogenic acids, and melanoidins

Caffeic, chlorogenic acids, and melanoidins concentrations are reported in Table 3. In general, EAE increased the caffeic acid and decreased the chlorogenic acid. Indeed, the highest concentration of caffeic acid was found in SCG-ME (2.22 ± 0.06 mg/g SCG) while of chlorogenic acid in SCG and SCG-C (2.20 ± 0.01 and 2.20 ± 0.13 mg/g SCG, respectively). The concentration of both acids was slightly but significantly higher in SCG-ME than SCG-E. Conversely, the concentration of melanoidins increased when MAE and EAE were applied to SCG. Indeed, the melanoidins concentration increased 7 folds (11.92 ± 0.02 and 72.27 ± 0.10 mg/g SCG in SCG and SCG-ME, respectively) demonstrating the additive effect of microwave and enzymes. The cryogenic milling slightly increased melanoidins concentration in SCG-C and SCG-CE compared to SCG and SCG-E, respectively (Table 3).

3.5. Antioxidant activity

The antioxidant activity of SCG extracts was evaluated by DPPH, ABTS, FRAP assays (Fig. 4).

The highest radical scavenging activity was found in SCG-ME with values of 89.21 ± 0.70 and 30.31 ± 0.18 μmol of Trolox/g of SCG in ABTS and DPPH assays, respectively, while 126.97 ± 4.14 μmol of $FeSO_4$ in FRAP assay, confirming how both microwave and enzymatic treatments increased the antioxidant properties of SCG. Indeed, SCG-M and SCG-E were the other samples with high antioxidant properties, with SCG-M with higher values in all three assays, but with no statistical differences for DPPH assay. The cryogenic milling did not increase the antioxidant properties of SCG, and no statistical differences were found between SCG and SCG-C and between SCG-E and SCG-CE.

3.6. Probiotic bacteria growth

The capability of 11 strains of probiotic microorganisms to grow on treated SCG was evaluated by two-steps consisting firstly in the selection of one probiotic microorganism able to growth in SCG or SCG-E extracts. No growth was observed in SCG while 5 strains were able to grow in SCG-E extract, namely *L. plantarum* LP01 and LP19, *B. animalis* B501, *La.*

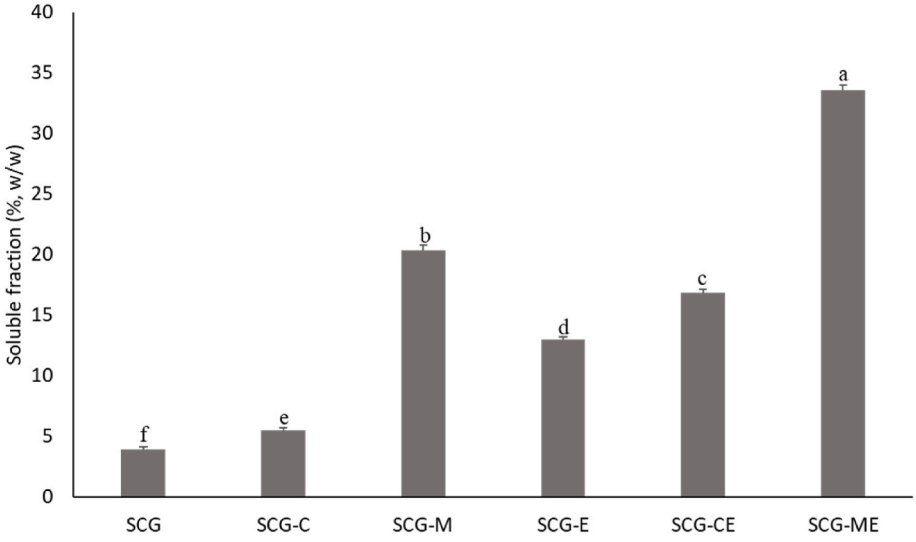

Fig. 2. Amount of soluble fraction released in water in SCG before any treatment, after cryogenic milling (SCG-C), MAE (SCG-M), EAE (SCG-E), combination of cryogenic milling and enzymatic treatment (SCG-CE), and combination of MAE and EAE (SCG-ME).

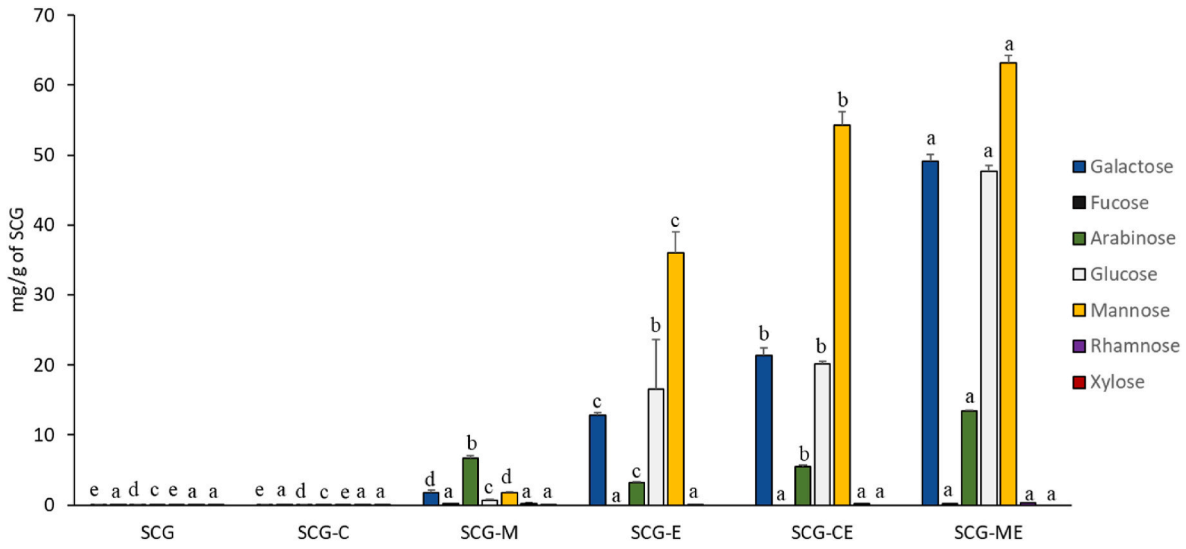

Fig. 3. Quantification of galactose, fucose, arabinose, glucose, mannose, rhamnose, and xylose in SCG before any treatment, after cryogenic milling (SCG-C), MAE (SCG-M), EAE (SCG-E), combination of cryogenic milling and EAE (SCG-CE), and combination of MAE and EAE (SCG-ME).

Table 3
Caffeic and chlorogenic acids, and melanoidins concentrations in SCG before any treatment, after cryogenic milling (SCG-C), MAE (SCG-M), EAE (SCG-E), combination of cryogenic milling and EAE (SCG-CE), and combination of MAE and EAE (SCG-ME).

	Caffeic acid (mg/g SCG)	Chlorogenic acid (mg/g SCG)	Melanoidins (mg/g SCG)
SCG	< LOQ	2.20 ± 0.01^{a}	11.92 ± 0.02^{e}
SCG-C	< LOQ	2.20 ± 0.13^{a}	14.87 ± 0.01^{d}
SCG-M	0.05 ± 0.04^{c}	1.97 ± 0.11^{b}	35.55 ± 0.16^{b}
SCG-E	1.73 ± 0.04^{b}	0.15 ± 0.02^{d}	32.37 ± 0.08^{c}
SCG-CE	1.96 ± 0.20^{a}	0.16 ± 0.01^{d}	33.00 ± 0.03^{c}
SCG-ME	2.22 ± 0.06^{a}	0.58 ± 0.11^{c}	72.27 ± 0.10^{a}

[a–c]Values in the same column with different superscript letters differ significantly ($p < 0.05$).

paracasei LPC2, and *Le. brevis* LB17. Four of them, *B. animalis* B501, *L. plantarum* LP19, *La. paracasei* LPC2, and *Le. brevis* LB17 showed the highest value of A ranged from 0.85 ± 0.04 to 0.88 ± 0.05 with no statistical differences (Supplementary Table 3). *L. plantarum* LP19 showed the lowest value of λ and the highest value of μ_{max} (2.67 ± 0.18 and 0.045 ± 0.003, respectively) (Supplementary Table 4), thus considering this strain as a suitable strain for the evaluation of probiotic growth on treated SCG.

Therefore, *L. plantarum* LP19 was used to ferment SCG, SCG-C, SCG-M, SCG-E, SCG-CE, and SCG-ME by evaluating the cell density after 24 h of incubation and the change in pH. The inoculum density was 7.14 ± 0.17 log10 cfu/g. No significant growth was found in SCG (7.09 ± 0.25 log10 cfu/g), SCG-C (7.11 ± 0.38 log10 cfu/g) and SCG-M (7.15 ± 0.21 log10 cfu/g) and a slight decrease of pH (from 0.18 ± 0.09 to 0.42 ± 0.13) was detected. Conversely, EAE led to the increase of cell density in SCG-E, SCG-CE, and SCG-ME which resulted from 8.99 ± 0.37 log10 cfu/g in SCG-CE to 9.22 ± 0.29 log10 cfu/g in SCG-E. The pH dropped in these samples to values lower than 4 for all the samples with the lowest in SCG-CE (3.77 ± 0.15).

4. Discussion

The SCG valorization has been widely exploited due to the high availability worldwide, the low cost, and the high concentrations of valuable compounds (Banu et al., 2021; Kourmentza, Economou, Tsafrakidou, & Kornaros, 2018), but requiring a stabilization process mainly due to the high-water activity which can increase the microbial contamination in few hours. Martinez-Saez et al. (2017) evaluated

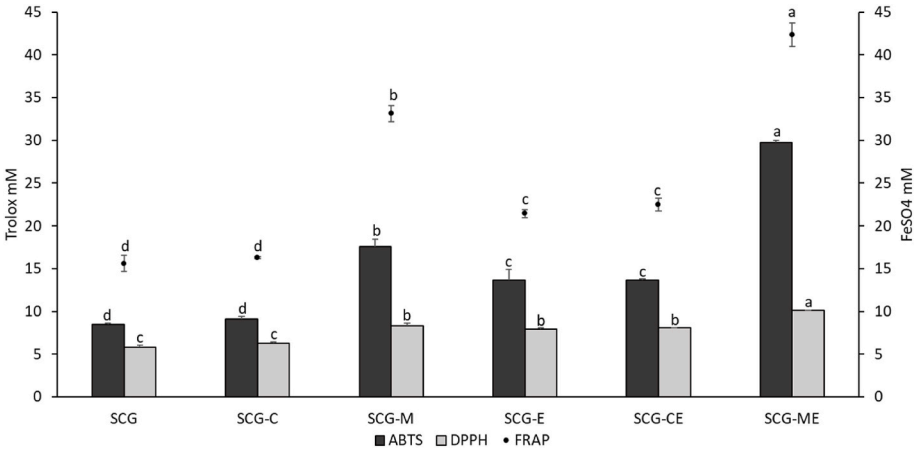

Fig. 4. Antioxidant properties of SCG before any treatment, after cryogenic milling (SCG-C), MAE (SCG-M), EAE (SCG-E), combination of cryogenic milling and EAE (SCG-CE), and combination of MAE and EAE (SCG-ME) evaluated by DPPH, ABTS, FRAP assays.

different stabilization processes finding the use of temperature between 40 and 70 °C as the optimal one. SCG is the residue of a hot water extraction of coffee and the scope of the treatments is to facilitate the extraction or the utilization of its component. Different methods were adopted to extract high values compounds, including physical, chemical, and biotechnological processes. In this work, a factorial design was setup aiming at the optimization of the enzymatic process for increasing the solubility of the insoluble fraction of SCG. Viscozyme®L and Celluclast®1.5L use was then optimized and applied in combination with MAE. The results were in line or superior to the literature as more than 40% of the insoluble fiber was solubilized using a combination of enzymes and MAE.

Among the enzymes used in this study, Viscozyme®L was previously used after microwave-pretreatment of SCG to create oleogels rich in bioactive chemicals (Mavria et al., 2022) and for producing non-digestible oligosaccharides (approximately 20% of sugar after enzymatic hydrolysis, roasting, and hot water infusion extraction) from SCG after green coffee production to test the prebiotic efficiency of the extract (Desai, Martha, Harohally, & Murthy, 2020). Celluclast®1.5L was used in combination with other processes for producing SGG hydrolysates rich in sugars (from 30 to 70% of sugars extracted from SCG) which could be used as a source of high value compound or as a substrate for producing biofuels or bioplastic (Battista et al., 2020; Nguyen, Cho, Lee, & Bae, 2019; Obruca, Benesova, Kucera, Petrik, & Marova, 2015). The MAE was previously used for the extraction of galactomannan oligosaccharides which were recovered in higher amount at 200 °C (Passos et al., 2013, 2019) reaching the extraction yield of 43% thus confirming the beneficial use of this technology for their solubilization.

The oligosaccharides from SCG were well characterized for their functional and prebiotic activity (Gu et al., 2020; Tian, Freeman, Corey, German, & Barile, 2017; Wongsiridetchai et al., 2021). Galactomannans consist of a linked 1,4 mannan chain with galactose ramification with different degrees of branching. They were recently considered as a novel class of oligosaccharides with potential prebiotic use (Jana, Suryawanshi, Prajapati, & Kango, 2021; Priya, 2020) and SCG could be considered a good source of these compounds, which represent more than 40% of the total carbohydrates in SCG (Jooste, García-Aparicio, Brienzo, Van Zyl, & Görgens, 2013). However, a decrease in oligosaccharides was found when combined MAE and EAE were applied (17 g/100g of total sugars). The presence of high concentrations of mannose and galactose in SCG-ME confirmed the breakage of the galactomannan polymers from insoluble fiber and the oligosaccharides released in SCG-M. Arabinose, probably derived from the breakage of arabinogalactan type II, slightly increased after EAE in SCG-M. Moreover, in accordance with the oligosaccharides quantification, the highest concentration of soluble fiber was found in SCG-M.

The quantification of melanoidins, chlorogenic and caffeic acids demonstrated that SCG is a significant source of bioactive substances. The overall phenolic acids concentration shown in SCG samples was approximately 2.20 mg/g, in accordance with Castaldo et al. (2021) and higher than those found by Angeloni et al. (2020). A very high variation in phenolic acids content is reported in literature, probably due to several parameters, among which SCG origin, brewing techniques, and roasting degree, are the most impactful (Tfouni et al., 2014). Chlorogenic acid is the most important phenolic acid in SCG and numerous epidemiological and experimental research correlated chlorogenic acid with important health properties also related to gut microbiota composition (Kanno et al., 2013; Mills et al., 2015; Tajik, Tajik, Mack, & Enck, 2017). A large fraction (approximately 65%) of the chlorogenic acids intake passes the small intestine and ends up in the colon where it is hydrolyzed by the gut microbiota using the quinic moiety as carbon source while caffeic acid is almost all directly adsorbed (Olthof, Hollman, & Katan, 2001). The quantities of melanoidins observed (approximately 7 g/100g in SCG-ME) were lower than those found in previously analyzed SCG samples, which reported a concentration range between 13 and 25 g/100 g (Castaldo et al., 2021; Mesías & Delgado-Andrade, 2017). A significant increase of melanoidins was found in SCG-ME suggesting that the hydrolysis of soluble fiber, which decreased in SCG-ME if compared to SCG-M, led to their further release in the extracts. Melanoidins are well known as heterogeneous polymer present in SCG material that can incorporate chlorogenic acid during coffee roasting and exert a significant antioxidant activity (Bekedam et al., 2006; Moreira, Nunes, Domingues, & Coimbra, 2012). The higher releases of these compounds in extracts after treatments was positively correlated with higher antioxidant properties tested by ABTS (0.98, $p = 0.0002$), DPPH (0.97, $p = 0.0014$), and FRAP (0.94, $p = 0.0057$) assays. Indeed, antioxidant properties of SCG extracts were always positively correlated to the different treatments. A positive correlation of 0.82 ($p = 0.0070$) was found between ABTS and DPPH assay, 0.93 ($p = 0.0002$) between DPPH and FRAP assays, and 0.92 ($p = 0.0004$) between ABTS and FRAP assays. The cryogenic milling did not increase the antioxidant properties of SCG in the water-soluble fraction, however reduced particle size could influence the antioxidant potential of SCG powder if evaluated by QUENCHER methodology as previously demonstrated in cereal-based products (Henrion, Servaes, Thielecke, & Fogliano, 2018). Moreover, melanoidins behave as dietary fibre and they are a very good substrate for microbial growth having known prebiotic function (Morales, Somoza, & Fogliano, 2012).

The optimized enzymatic hydrolysis was found as effective tool to increase the fermentability of SCG, resulting in a good growth substrate for these probiotic strains and especially for *L. plantarum* LP19 which showed the lowest phase, and the highest growth rate speed and increase in cell densities after 24 h of fermentation. The high release of oligosaccharides and monosaccharides after MAE and EAE, respectively, and the high release of melanoidins acted as growth promoters for the probiotic microorganism. Lactic acid bacteria are part of coffee microbiota (de Melo Pereira et al., 2020) and in particular *L. plantarum* is a well-known lactic acid bacterium widely characterized for its tolerance to high levels of phenolics and ability to adapt its metabolism to different stress conditions (Filannino, Di Cagno, & Gobbetti, 2018; Papadimitriou et al., 2016). The treatments combination and the proper selection of bacterial strain applied in this work demonstrated the possibility to overcome the difficulties of using SCG as a substrate for microorganisms' growth due to the presence of inhibitors (Kovalcik et al., 2018). The possibility to include probiotic microorganisms in ingredients or products formulations with prebiotic potentials has been widely investigated in the last years to produce synbiontic with positive impact on people health (Palai, Derecho, Kesh, Egbuna, & Onyeike., 2020; Swanson et al., 2020). The high cell density of *L. plantarum* LP19 at the end of fermentation and the increase in oligosaccharides and soluble fiber in treated SCG suggest a possible use as symbiotic.

In this work the use of commercial enzymes and microorganisms was evaluated aiming at the application in food products without evaluating the potential impact of storage conditions and possible degradation of extracts. The incorporation of extracts in various products requires a case-by-case examination considering the complexity of food matrices.

5. Conclusions

In this work, an improved release of bioactive compounds from SCG was achieved by means of different technological approaches. Physical and biotechnological processes were applied to increase the functional potential of the matrix enriching the soluble fiber, oligosaccharides, sugars, melanoidins, and phenolic acids contents. The use of MAE increased the soluble fiber and oligosaccharides while EAE increased the sugar contents, the conversion of chlorogenic acid into caffeic acid, and the possibility to use SGC as a growth substrate for probiotic microorganism. Moreover, the combination of these two treatments led to a further increase of sugars and the antioxidant potential of the extracts compared to a single treatment process. For the first time, novel options for SCG valorization were investigated and developed at lab scale by

combining *green* approaches (e.g. use of commercial enzymes and selected microorganisms), and conventional technologies. A future industrialization of the processes proposed in this work should include a detailed evaluation of the cost implications, which however depend on the processing flows and the type and the extent of functionality desired.

CRediT authorship contribution statement

Marco Montemurro: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Melania Casertano:** Formal analysis, Writing – review & editing. **Arnau Vilas-Franquesa:** Formal analysis, Writing – review & editing. **Carlo Giuseppe Rizzello:** Conceptualization, Writing – review & editing. **Vincenzo Fogliano:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.115974.

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