A Sustainable Journey with Coffee Silverskin from Agricultural Waste to Emerging Roles in Functional Foods, Bioconversion Processes, Advanced Functional Coatings, and Eco-Friendly Textile Applications

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Ph.D. in Food Science

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Chapter II *Safety assessment in food sector*

Coffee Silverskin: Chemical and Biological Risk Assessment and Health Profile for Its Potential Use in Functional Foods

Brief overview and structure of the thesis

In the ever-changing landscape of sustainable practices, exploring alternative uses for waste from the agri-food supply chain has become a focal point for researchers and innovators. The paradigm of the circular economy fits into this context as a key reading in converting the linear and consumerist approach of resources to a more circular model, in which waste is minimized and materials reused or recycled. Researchers and innovators are increasingly attracted to the prospect of transforming what was once considered a simple by-product into valuable resources.

It is this fresh perspective that inspired the development of the following doctoral project, embarked upon as a sustainability journey by elevating the role of Coffee Silverskin (CS) – a thin protective layer of the coffee bean that detaches from it during the roasting phase and is often considered a waste material.

This doctoral thesis aims to deepen the sustainable potential of this by-product of the agri-food chain by evaluating its application potential in multiple sectors. The thesis is structured into seven chapters, each centered around the identification of potential (re)uses for this waste by-product within the coffee supply chain.

Chapter I offers an in-depth exploration of the essential attributes of Coffee Silverskin. The study provides a comprehensive characterization of CS to evaluate its chemical profile and nutritional value, with a focus on the content of bioactive peptides, and a thorough analysis of its physical structure. The results obtained show its usefulness in the areas of nutrition, nutraceuticals, and industrial applications.

Chapter II assesses its potential in the food sector for the development of functional foods, given its high fibre content and complex nutritional profile. To this end, a detailed investigation of the toxic elements, rare earth elements (REE), pesticides, polycyclic aromatic hydrocarbons (PAHs) and micro-organisms was provided on the two main varieties of coffee, *Arabica* and *Robusta*, and their mixture. The risk assessment, based on the potential consumption of CS as a food ingredient, did not reveal any non-carcinogenic or carcinogenic risk. In conclusion, this study provides the first evidence of the safety of CS based on determinist assessment.

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Chapter VI is dedicated to the development of valuable biomaterials, utilizing Coffee Silverskin as an ethical alternative to leather and synthetic materials in the production of textile coatings. The innovative exploration of CS as a bio-based coating material encompasses various residue treatment methods and formulations, aiming to evaluate its viability as a more sustainable option. Through these assessments, the impact of utilizing planetary ball milling equipment as a pre-treatment method has resulted in a homogeneous deconstruction of the integument, yielding a uniform and soft coating. Leveraging the potential of coffee silverskin enables the textile industry to play a role in fostering a more sustainable and responsible future.

Aims and thesis outline

- ✓ **Characterization and possible utilizations**: the thesis aims to explore and identify potential (re)uses for Coffee Silverskin (CS) in various sectors, emphasizing its versatile applications in nutrition, nutraceuticals, industrial settings, and more.
- ✓ **Safety assessment in food sector**: conduct a comprehensive assessment of CS's potential in the food sector, examining its high fiber content and complex nutritional profile. Assess the safety of CS as a food ingredient through risk assessments.
- ✓ **Sustainable waste management**: investigate sustainable waste management by utilizing black soldier fly larvae (BSFL) for the bioconversion of CS. The research aims to reduce by-product waste and generate high-protein insect biomass.
- ✓ **Innovative coating for food-packaging solutions**: Introduce a new mechano-chemical process to upcycle CS into thin bio-coatings for flexible packaging, offering a sustainable alternative and contributing to waste reduction.
- ✓ **Sustainable new material in textile**: Address environmental challenges in the textile industry by exploring CS as a bio-based coating material for textiles and contributing to a more eco-friendly fashion sector.

Overall, the thesis aims to contribute to a more sustainable future by exploring innovative uses for CS across different industries and applications.

Valorization Of Coffee Industry Wastes: Comprehensive Physicochemical Characterization Of Coffee Silverskin And Multipurpose Recycling Applications

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Abstract

Every year, the coffee supply chain produces tons of waste materials from the processing of the coffee bean. Coffee silverskin (CS) is the only waste product from the coffee bean roasting phases but as roasting is a key step in obtaining the coffee beverage, there are tons of CS collected and thrown away by coffee roasting industries. This study is based on the characterization of this integument, with a view to its use in nutritional, nutraceutical and industrial fields. From the results obtained, silverskin can be used in the food sector for its nutritional profile with a good amount of protein (18.9%) and total dietary fiber (34.7%) and its low-fat content (3.0%). Food safety was also determined by evaluating the low levels of contaminants such as ochratoxin A (OTA) and acrylamide (AA), with values <0.1 mg/kg and <0.02 mg/kg respectively. Low concentrations of heavy metals and polycyclic aromatic hydrocarbons (PAHs) completed the safety-related overview in terms of CS contamination. For the nutraceutical aspect, an in-silico analysis of the peptide fraction was proper to evaluate the presence of angiotensin-converting enzyme (ACE) inhibitory peptides, antioxidants and dipeptidyl peptidase (DPP) IV inhibitors with promising uses for the treatment of diabetes and its side effects. Finally, through morphological-structural characterization of CS, the main constituents such as lignin, cellulose and hemicellulose were identified spectrally by Fourier transform infrared spectroscopy (FT-IR) and solid-state CP/MAS 13C NMR spectroscopy. The CS thermostability was monitored by thermogravimetric (TG) analysis, and the tegument surface was evaluated by Scanning Electron Microscopy (SEM) and volumetric nitrogen adsorption analysis. This information provides a broader view of the possible uses of CS as a filler for bio-composite materials. Therefore, from the perspective of recovering waste materials from food supply chains, coffee silverskin could be a good product to focus on.

Keywords: Silverskin; coffee by-products; recycling; integument; agri-food waste.

1. Introduction

The growing attention to environmental protection and circular economy has encouraged the study and exploitation of waste materials from the food industry. Tonnes of by-products are discarded every year without considering their enormous potential for the realization of new products or structural and qualitative improvement of products already on the market. The Food Agriculture Organization of the United Nations (FAO) has set a priority for implementing solutions for more competitive, inclusive, and sustainable food value chains that involve optimized supply chains and circular bioeconomy for loss and waste reduction. Coffee, for example, produces a huge amount of waste products throughout the various stages of processing. The structure of the freshly harvested coffee cherry consists of a series of protective layers of the coffee bean (Figures 1), which are discarded during the early stages of processing. About 90% of the coffee cherry is discarded and often inappropriately disposed of, causing environmental problems (Iriondo-DeHond et al., 2020b). In relation to their chemical and physical composition, it has been noted that some by-products are more suitable for the production of edible mushrooms and vermicompost (coffee pulp and husk, respectively), whereas silverskin has potential in the food sector as a dietary fiber supplement and spent ground coffee for biofuel production (Murthy and Naidu, 2012; Janissen and Huynh, 2018).

The following study focuses on coffee silverskin (CS), a thin integument covering both hemispheres of the green coffee bean and separating from it during the roasting phase (Figure 2). It represents only 4% (w/w) of the coffee bean (Martuscelli et al., 2021) but, compared to the 10 million tonnes of coffee produced annually (ICO, 2020), an amount of 400 thousand tonnes of waste products are generated each year in the coffee roasting industry. Recent studies investigated the recycling potential of this integument to be used in the food industry

as a novel food low in fat, high in fiber and a source of protein (Iriondo-DeHond et al., 2019a, 2019b; Klingel et al., 2020; Martuscelli et al., 2021); in the biorefineries as biomass for biobutanol production (Hijosa-Valsero et al., 2018); as potential material for packaging (Garcia and Kim, 2021; Oliveira et al., 2021) but also in the nutraceutical and cosmetic field for its bioactive compounds (such as phenolic compounds and melanoidins) with antioxidant properties (del Pozo et al., 2020; Nzekoue et al., 2020; Zengin et al., 2020). For a complete characterization of this waste material, our study aims to provide additional helpful information on the peptide fraction of CS extract and fill some gaps in the structural analysis of this byproduct. To the best of our knowledge, there are few studies conducted on CS for the determination of the peptide composition of protein hydrolysates and their potential bioactivities (Perez-Miguez et al., 2019). A recent study conducted by Górska et al., 2020 on the characterization of thermal properties of CS by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) under nitrogen and oxygen flow.

Our work takes a broad perspective on CS characterization, with the primary objective: i) to confirm the nutritional profile and safety of CS to make a healthy food product; ii) to study the peptide composition of CS and its biological activities, to ascertain possible uses in nutraceuticals as conventional drugs for the treatment of diabetes; and iii) to characterize its structure on macro- and micro-scale with the aim of possible use as a possible filler for the realization of polymeric composite material. This research may open up to new opportunities for using this food waste material for environmentally sustainable recycling that aims to minimize waste within the coffee production chain and provide the basis for the production of increasingly environmentally friendly products.

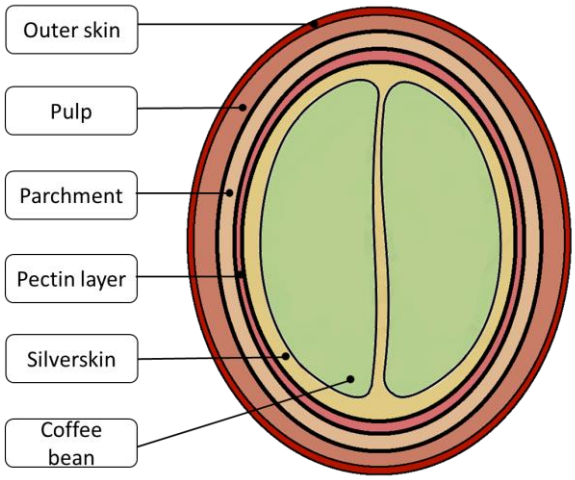

Figure 1. Identification of the different layers that constitute coffee cherries.

Figure 2. Coffee silverskin, the only waste material from the roasting phase of the green coffee beans.

2. Materials and methods

2.1. Coffee Silverskin sample

Two coffee industries of the Campania region (Italy) supplied the thin integument just after the roasting process of *Coffea arabica* and *Coffea canephora* varieties (in unspecified ratio). Coffee varieties are usually blended by the industries during the roasting process, and the coffee silverskin was recovered by suction cyclones and collected (Lachenmeier et al., 2021). The samples were stored in the dark at 20 °C and eventually analyzed within three days, in triplicate.

2.2. Nutritional profile and safety aspects

Physico-chemical property analyses were performed on coffee silverskin samples to evaluate their nutritional profile and healthiness in terms of the absence of contaminants. To this end, the analyses were carried out as described in the following sections.

2.2.1. Protein fraction

Determination of the protein fraction was performed according to the Kjeldahl procedure, which measures the nitrogen content of a sample (ISTISAN Report 96/34). Protein content was calculated by assuming a ratio of protein to nitrogen for the CS sample analyzed. Briefly, 0.1 g of ground CS was placed in the digestion tube. To this, one catalyst tablet and 7 ml of sulfuric acid were added. After the sample completed digestion, the digestate was diluted thoroughly with deionized water and the sample in the tube was shaken. Next, an appropriate volume of boric acid solution was added to the collection flask. The sample tube was immersed in the boric acid solution and distillation was proceeded to completion. Finally,

the sample was titrated with the standardized HCl solution. CS samples were prepared in triplicate.

2.2.2. Total fat

The total fat determination was performed by Soxhlet following the official method ISTISAN report 96/34 (Baldini et al., 1996). 5 g of CS powder was weighed and placed in a cellulose extraction thimble. The thimble was placed in the Soxhlet extractor for 3 hours with petroleum ether. The solvent was removed by distillation with a rotary evaporator, and the residue was dried in an oven at 100 °C for 1 hour. The extracted sample was allowed to cool in a desiccator and weighed.

2.2.3. Saturated fatty acids

The fatty substance, appropriately extracted from the CS sample, was subjected to transesterification, and the fatty acid methyl esters, thus obtained, were analyzed by gas chromatography. Briefly, 100 mg of sample oil into a vial with a tight sealing cap, adding 5 mL of hexane. Then, the vial was vortexed, 250 µl of sodium methoxide reagent was added. After, it was vortex again for 1 min, pausing every 10 second. 5 mL of saturated NaCl solution was added and manually shake vigorously for 15 s. Let stand for 10 min. The sample was stand for 10 minutes and then, the hexane layer was removed and transferred to a vial containing a small amount of Na2SO4. After 15 min the hexane phase was again transferred to a vial for subsequent GC analysis.

2.2.4. Carbohydrates

10 g of finely ground sample was extracted with 100 ml of the acetonitrile/water mixture (60:40) in an ultrasonic bath for 30 min. The sample was stirred for 3 hours and the

extract was injected into high-performance liquid chromatography (HPLC). Separation and determination are performed by HPLC using a specific chromatographic column; detection is by refractive index detector, or UV detector.

2.2.5. Total dietary fiber

Total dietary fiber was determined with the Megazyme Integrated Total Dietary Fiber Assay kit, which was accepted as AOAC method 2017.16 in 2018 and is the only method consistent with the CODEX Alimentarius definition of dietary fiber (Martuscelli et al., 2021). Enzymatic digestion with heat-stable alpha-amylase, protease and amyloglucosidase was performed to remove protein and starch. Next, precipitation with ethanol was carried out, and the residue was filtered and dried. The resulting mass, minus the remaining ash and protein, represents the amount of total dietary fiber.

2.2.6. Ochratoxin A (OTA)

The determination of ochratoxin A (OTA) in the CS was performed following the official method EN 1413:2003 with some modifications. 10 g of sample were homogenized with methanol:water solution (70:30 v/v); the extract was diluted with a phosphate buffer solution prepared by dissolving in 1800 mL of water 4.0 g of KCl, 160g of NaCl and 36.0g of sodium hydrogen phosphate dihydrate (Na_2HPO_4-$2H_2O$), adjusting the pH of the solution to 7.4 with 0.1 M HCl or with 0.1 M NaOH. Purification was then performed by immunoaffinity chromatography onto columns containing a gel on which anti-OTA antibodies are immobilized. Finally, mycotoxin eluted with pure methanol was quantified by HPLC with a reversed-phase C18 column and a fluorimetric spectrum detector.

2.2.7. Acrylamide (AA)

Analyses for acrylamide (AA) were performed according to the method of Fernandes and Soares (2007) with some modifications. An aliquot of 0.5 g of homogenized sample was subjected to a solid-phase extraction technique dispersed with C18 sorbent packed in polypropylene columns. The aqueous eluate was then subjected to derivatization after bromination and subsequent GC-MS reading and quantification of the derivative.

2.2.8. Furan

The furan was analyzed with head space technique following the Park et al., 2021 method. An aliquot of 5 g of sample and 5 mL of HPLC water were added to a headspace vial (20-mL) carefully sealed. Then d4-furan solution (internal standard) was added to vial, homogenised and placed on ice. Furan was extracted from the samples by an automated solid-phase micro-extraction (SPME) agitating the sample at 300 rpm, 50°C. The fibre (carboxen/polydimethylsiloxane) was exposed to the headspace of vial samples for 20 min at 25 mm depth. Then, the fibre was thermally desorbed in the injection port of a gas chromatograph at 40 mm depth for 5 min.

2.2.9. Heavy metal

A 0.5 g aliquot of CS was homogenized using a blender, and 5 ml of 65% HNO_3 and 2 ml of H_2O_2 (30% w/w) were added. Then, the sample underwent wet mineralization via a Milestone microwave for 30 min at 190°C. Eventually, the sample was cooled, and the final volume of 25.0 ml was obtained by adding MilliQ water. Trace element detection and quantification were performed using a Thermo Scientific TM ICAPTM RQ inductively coupled plasma mass spectrometer (Q-ICP-MS) with a Burgener Mira-Mist nebulizer, a

quartz cyclonic spray chamber cooled to 2.7°C, and foam cones. All samples were analyzed in duplicate, and each sample was measured in triplicate with Q-ICP-MS detection.

2.2.10. Polycyclic Aromatic Hydrocarbons (PAHs)

PAH analyses were performed with acetone (≥99.8%, GC grade) and n-hexane (≥95%, GC grade). Certified multicomponent solution of 12 PAHs (2000 µg/mL each) and deuterated internal standard mixture solution (100 µg/mL each) were provided by Ultrascientific (Italy). All glassware was tested and found to be free of PAHs. An aliquot of 1.00 g ± 1 mg CS sample was extracted with 25 mL acetone/n-hexane 1:1 v/v using ultrasonic bath (Branson, US) for three 30-minutes cycles, in closed glass vials. After this time, the extract was concentrated to a final volume of 1 mL using automatic concentrator MultiVap 8 (Labtech, Italy) and purification procedure was unnecessary for the subsequent GC-MS analysis. The extract was injected into a gas chromatograph coupled to a mass spectrometer (GC 2010 Plus, MS-TQ8030, Shimadzu, Japan) for the determination of PAHs (Arienzo et al., 2017). Calibration curves were obtained in the range of 1 to 100 µg/L by calculating the area ratio of the target ion to the respective internal standard with five standard solutions obtained from certified standard solutions. A reagent blank and a digestion method blank were run to verify contamination of all materials, and calibration was verified in the instrumental sequence with a control standard every 10 samples.

2.3. Nutraceutical aspects

2.3.1. Silverskin Protein Isolate (SPI)

Proteins of CS were isolated by adapting isoelectric precipitation technologically used for soy proteins and hemp proteins (Mamone et al., 2019). Briefly, 10 g of CS flour were

mixed with 100 ml of deionized water adjusted to pH 10.0 with sodium hydroxide 0.1 N. The mixture was stirred for 4 hours at 35 °C on a magnetic stirrer. After 4 h extraction, samples were centrifuged at 3433 g for 15 min, and the supernatants were retained. The supernatants were adjusted to pH 4.5 with HCl 0.1N and put in the refrigerator overnight to enhance the protein precipitation. Then the precipitate was collected by centrifugation for 15 minutes at 3433 g. The pellets were washed twice in cold acetone and dried with gaseous nitrogen. To evaluate the presence of soluble peptides 10 mg of SPI were dissolved in TFA 0.1% (v/v) and centrifugated. The supernatant was cleaned up using C-18 Sep-Pak cartridges (Waters, Milford, Massachusetts, USA) following the manufacturer's instruction and then concentrated with gaseous nitrogen and resuspended in 0.1% (v/v) formic acid solution. The peptide mixtures were finally analyzed through high-resolution mass spectrometry.

2.3.2. High-resolution liquid chromatography (HR LC-MS/MS) analyses

Mass spectrometry (MS) analysis was performed by using a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA), online coupled with an Ultimate 3000 ultra-high- resolution liquid chromatography instrument (Thermo Scientific). Samples were loaded through a 5mm long, 300 μm id pre-column (LC Packings, USA) and separated by an EASY-Spray™ PepMap C18 column (2 μm, 15 cm×75 μm) 3 μm particles, 100 Å pore size (Thermo Scientific). Eluent A was 0.1% formic acid (v/v) in Milli-Q water; eluent B was 0.1% formic acid (v/v) in acetonitrile. The column was equilibrated at 5% B. The peptides generated after the hydrolysis of the spots were separated by applying a 5–40% gradient of B over 60 min. The flow rate was 300 μL/min. The mass spectrometer operated in data-dependent mode and all MS1 spectra were acquired in the positive ionization mode with an m/z scan range of 350-1600. Up to 10 most intense ions in MS1 were selected for MS/MS

mode fragmentation. Resolving power of 70,000 full width at half maximum (FWHM), an automatic gain control (AGC) target of 1×106 ions and a maximum ion injection time (IT) of 256 ms were set to generate precursor spectra. MS/MS fragmentation spectra were obtained at a resolving power of 17,500 FWHM. In order to prevent repeated fragmentation of the most abundant ions, a dynamic exclusion of 10s was applied. Ions with one or more than six charges were excluded. Spectra were processed by using the Xcalibur Software 3.1 version (Thermo Scientific) and then analyzed using the ProteinProspector software (v 6.2.2. https://prospector.ucsf.edu/prospector/mshome.htm). The analysis of the mass spectra was performed using as reference proteome all the "Green plants" plant sequence data (UniProtKB.2020.09.02.random.concat). The database searching parameters were: i) mass tolerance value of 8 ppm for the precursor ion and 0.01 Da for MS/MS fragments; ii) no enzyme iii) no fixed modification; variable modifications: oxidation of the methionine and pyroglutamic acid for the N-term glutamine residues. The false discovery rate was set at 1% on both peptides and proteins. All the entries inferred to fragments were removed in order to obtain a more reliable analysis. The analyses were performed on biological duplicates, and the results were finally merged removing duplicates. The resulting peptides were searched for their potential bioactivity through the Biopep software (Minkiewicz et al., 2019). This online tool (available on https://biochemia.uwm.edu.pl/about-biopep-uwm/ - last access on 15/02/2022) is a curated database of bioactive sequences. For angiotensin-converting-enzyme (ACE) and dipeptidyl peptidase IV (DPP IV) inhibitory activities, only bioactive sequences contained at least ten times were reported.

2.4. Technical-structural characterization

2.4.1. Fourier Transform Infrared (FTIR) spectroscopy

Fourier-transform infrared spectra were recorded in Attenuated Total Reflectance (ATR-FTIR) mode on a Perkin Elmer Spectrum 100 spectrometer equipped with a Universal ATR cell (Perkin Elmer Inc., Waltham, MA, USA). The scanned wavenumber range was 4000–650 cm^{-1}. All spectra were recorded with a resolution of 4 cm^{-1}, averaging 16 scans.

2.4.2. Solid-state Cross-Polarization/Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CP/MAS 13C NMR) spectroscopy

Solid-state 13C NMR spectra recorded in cross-polarization, magic angle spinning mode (CP-MAS) were collected at 100.47 MHz on a Bruker Avance II 400 spectrometer (Bruker Biospin Co., Billerica, MA,USA) operating at a static field of 9.4 T, equipped with a 4 mm MAS probe. CS samples were packed in 4 mm zirconia rotors sealed with Kel-F caps and spun at 10 kHz. Spectra were recorded with a 1H $\pi/2$ pulse width of 3.9 μs, a 1H-13C contact time of 2 ms and a high-power proton decoupling pulse scheme during acquisition, averaging 20000 scans.

2.4.3. Thermogravimetric (TG) analysis

The thermogravimetric analysis was used to evaluate the thermal stability of the material. TG analysis was performed using a Perkin Elmer Pyris 1 thermobalance (Perkin Elmer). Briefly, 3.5 mg of sample was inserted in an alumina crucible and heated from room temperature to 800°C. The measurement was performed under an inert atmosphere (nitrogen) with a heating rate of 10°C/min.

2.4.4. Scanning Electron Microscopy (SEM)

SEM analysis was performed on the CS surfaces using FEI Quanta 200 FEG SEM (FEI, Eindhoven, The Netherlands) in high vacuum mode. Before analysis, the samples were sputter-coated with a thin layer of Au/Pd.

2.4.5. Volumetric nitrogen adsorption analysis

Volumetric nitrogen adsorption analysis at 77 K was performed on CS through a Micromeritics ASAP 2020 analyzer (Micromeritics Instrument Corporation, Norcross, GA, USA) using high purity gases (>99.999%). Before analysis, the sample was degassed at 120 °C for 10 h under vacuum ($p<10^{-5}$ mbar). Nitrogen adsorption analysis was performed up to 1 bar, and the result was plotted as the volume of nitrogen adsorbed per gram of material versus the relative pressure p/p^0, where p represents the N_2 absolute pressure and p^0 represents the saturation pressure of N_2 at the temperature of analysis. The Brunauer–Emmett–Teller (BET) specific surface area (SSA) of CS was determined by the linear part of the BET equation applied to the N_2 adsorption isotherm. Nonlocal Density Functional Theory (NLDFT) was applied to nitrogen adsorption isotherm to evaluate the pore size distribution of CS.

3. Results and discussion

3.1. Nutritional profile and safety aspects

The nutritional profile is the most studied aspect of CS. Hence, the first part of the study focused on the determination of protein fraction, carbohydrates, total fat, saturated fatty acids, , and total dietary fibers (Table 1). The content of proteins (18.9%), carbohydrates (42.0%), total fat (3.0%), total dietary fibres (34.7%) are in agreement with Narita and Inouye, (2014) and Ateş and Elmacı, (2019). Some differences could be attributed to the different extraction

methods used for the analysis, temperature of the water during the extraction process, and varieties of coffee beans. A study conducted by Bessada et al, (2018) showed that there is a significant difference on the chemical composition of silverskin obtained from *Coffea canephora* beans of different geographical parts (Brazil, Uganda, Vietnam, Cameroon, Indonesia and India). In particular, fatty acid composition and antioxidant profile played an important role in geographically differentiating coffee silverskin (Bessada et al., 2018). For the evaluation related to the safety for food purposes of this integument, the levels of Ochratoxin A (OTA) and Acrylamide (AA), heavy metals and Polycyclic Aromatic Hydrocarbons (PAHs) were performed. The results are shown in Table 1. As also specified by a recent study conducted by Iriondo-DeHond et al., (2020b) the main limitations to the use of this by-product resulting from the coffee roasting phase could be related to a possible high contamination by OTA and AA.

Ochratoxin A (OTA) is a widespread mycotoxin produced primarily by the fungi *Aspergillus* and *Penicillum* species (Malir et al., 2016). Its presence is usually associated with the storage of grain products and other plant-derived products such as herbs, spices, and even coffee beans (Petzinger and Ziegler, 2000). A study conducted by Romani et al., (2003) evaluated how the concentration of OTA even in highly contaminated coffee samples is reduced by 90% when subjected to the typical roasting of Italian espresso coffee. In any case, despite the high toxic risk caused by the presence of this mycotoxin, a recent study by Heintz et al. (2021) shows, through a Margin of Exposure analysis (MOE), that measured levels of OTA in coffee pose only a low risk to the consumer following single or repeated exposures.

Acrylamide (AA), on the other hand, is a toxic substance induced by the cooking of foods at high temperatures (Rifai and Saleh, 2020). Its formation during the Maillard reaction is triggered by the presence of reducing sugars and amino acids (Esposito et al., 2017). French

fries, cookies, bread, and roasted coffee are food products with higher concentrations of AA (Başaran et al., 2020; Esposito et al., 2021; Başaran et al., 2022). The accredited neurological and carcinogenic toxicity on animals has placed this substance as potentially carcinogenic for humans (group 2A), imposing limits on its content in food (IARC, 1994). A study conducted by Esposito et al. (2020) shows that at certain time-temperature conditions reached during coffee bean roasting, a decrease in AA content is obtained without affecting taste and aroma. Furan has also been classified by the International Agency for Research on Cancer as a Group 2B carcinogen, that is, possibly carcinogenic to humans (IARC, 1995). It is a heterocyclic organic compound produced typically through the Maillard reaction (Anese and Suman, 2013) and its formation is affected by temperature and pH conditions (Seok and Lee, 2020).

Regarding roasted coffee beans, EU Regulations 1881/2006 and 2017/2158 prescribe occurrence limits for ochratoxin A (OTA) and acrylamide (AA), 5 μg/kg and 400 μg/kg, respectively. Given the origin of the silver skin as a byproduct of the coffee roasting stage, trace amounts of these contaminants could be present. However, the results of our analysis report levels of OTA and AA below the threshold limit (Table 2), as previously reported in studies conducted by Iriondo-DeHond et al., (2019a) and Martuscelli et al., (2021). In addition, a broader screening was conducted to assess the concentration of polycyclic aromatic hydrocarbons (PAHs), heavy metals, and micronutrients (Table 2). The results obtained for PAHs and heavy metals could be safe for the consumer considering a reasonable consumption of CS as food ingredient (Nolasco et al. Unpublished results). A previous study conducted by Ballesteros et al., (2014) found higher concentrations for Cd (<0.15 mg/kg), Cr (1.59 mg/kg) and similar values for Ni (1.64 mg/kg) and Pb (<1.60 mg/kg). In contrast, Nzekoue et al. (2022) reported lower levels for Pb (0.3 mg/kg), Ni (0.5 mg/kg), Cr (0.23 mg/kg), and higher values for Hg (0.05 mg/kg) and As (0.1 mg/kg).

Among the essential elements, the iron (Fe) content is noteworthy. Its level (349 mg/kg) suggests a practical use of CS as a supplement to the daily requirement of this micronutrient. A use >12% (w/w) of CS as a food or food ingredient could receive the nutritional claim "high in iron" according to Regulation (EC) 1924/2006 and Regulation (EU) 1169/2011. An amount of 20g CS provides about 7 mg of iron, corresponding to 50% of the recommended daily allowance (RDA). Therefore, given its nutritional profile and food safety, the use of CS in the food sector could play an essential role as a food resource. In the context of the circular economy, the reuse of food waste is consistent with good management practices in the supply chain itself and allows for the improvement of the daily human diet with products of nutritional value. Thanks to the CS fiber content, its inclusion in the food diet could be useful to get the fiber intake recommended by the Italian Society of Human Nutrition (SINU) of 25 g/day of fiber for adults. In addition, the consumption of fiber-rich foods may improve body weight maintenance and reduce the risk of some diseases such as cardiovascular disease, type 2 diabetes and colon cancer (EFSA, 2010). However, it will be necessary to consider its safety by conducting an assessment of dietary exposure to coffee silverskin.

Table 1: Nutritional profile of coffee silverskin

Parameter	***Values***	***Units of Measurement***
Protein fraction	18.9	%
Carbohydrates	42.0	%
Total fat	3.0	%
Saturated fatty acids	1.2	%
Total dietary fiber	34.7	%

Table 2: Levels of toxicants and elements in coffee silverskin

Policyclic Aromatic Hydrocarbons (PAHs)	*Median concentration (mg/kg)*
Benzo(a)pyrene	< 0.01
Benzo(b)fluoranthene	0.01
Benzo(k)fluoranthene	< 0.01
Chrysene	< 0.01
Dibenzo-Pyrene	0.86
Dibenzo Anthracene	0.02
Pyrene	0.02
Perylene	< 0.01
Naphthalene	0.04
Acenaphthene	0.06
Fluorene	0.03
Fluoranthene	0.02
Process Contaminants	*Median concentration (mg/kg)*
Acrylamide (AA)	< 0.02
Furan	< 0.1
Methyl-furan	< 0.1
Mycotoxins	*Median concentration (mg/kg)*
Ochratoxin A (OTA)	< 0.1
Heavy Metals	*Median concentration (mg/kg)*
Arsenic (As)	0.04
Cadmium (Cd)	0.05
Total Chromium (Cr)	0.86
Mercury (Hg)	0.02
Nickel (Ni)	1.78
Lead (Pb)	2.63
Micronutrients	*Median concentration (mg/kg)*
Manganese (Mn)	22.9
Copper (Cu)	58.6
Cobalt (Co)	1.02
Zinc (Zn)	7.08
Iron (Fe)	349

3.2. Nutraceutical aspects

The chemical profile of CS is also interesting from the point of view of bioactive compounds. It is characterized by caffeine (0.77-1.4%), chlorogenic acids (15.8%) and melanoidins (17-23%), responsible for several biological activities, including antioxidant (Toschi et al., 2014; Ateş and Elmaci, 2019; Iriondo-DeHond et al., 2017; Gottstein et al., 2021). Several studies highlight the potential nutraceutical aspect of this integument (Nzekoue et al., 2020, 2022). The second objective of our work focused on the evaluation of the peptide fraction of CS and its potential benefits on human health.

The MS analysis showed the presence of 45 peptides inferred to protein involved in several metabolic pathways (Table 3 and Table 4); among these, serine proteases, carboxypeptidase and neprosin. The latter is a prolyl endoprotease that preferentially cleaves C-terminal to proline residues under highly acidic conditions and has been isolated and used as an alternative to the canonical trypsin in bottom-up proteomics (Schräder et al., 2017). The presence of these proteases could be associated with a potential positive action of CS extracts in the digestion process. Furthermore, in Table 3 it is possible to notice the presence of proteins involved in the oxidation of polyphenols such as tyrosinases and phenol oxidases. It is also noteworthy the presence of proteins involved in carbohydrate metabolism like glycoside hydrolase and alpha-mannosidase.

Table 3: Mass spectrometry (MS) analysis of protein found in the aqueous extract of coffee silverskin protein isolate (SPI). Uniprot ID: is the identification code in the UniprotKB protein database. An expectation value (E-value) represents a score that is proportional to the identification confidence of the peptide/protein.

Uniprot ID	E-value	Peptides	Taxonomy	Protein Name
A0A068UNL8	1.30E-12	15	*Coffea canephora*	Serine protease
A0A068UJ50	1.80E-08	7	*Coffea canephora*	Neprosin
A0A068VNV4	3.40E-09	5	*Coffea canephora*	Tyrosinase_Cu-bd domain-containing protein
A0A068UEN7	2.20E-09	3	*Coffea canephora*	Tyrosinase_Cu-bd domain-containing protein
M0SIL8	1.40E-11	3	*Musa malaccensis*	Neprosin
A0A068UNA6	2.10E-11	2	*Coffea canephora*	Serine protease
A0A218WZL2	2.20E-09	2	*Punica granatum*	Polyphenol oxidase, chloroplastic-like
A0A068V8L2	6.70E-06	2	*Coffea canephora*	Glycoside_hydrolase_SF
B9N1V7	6.10E-07	1	*Populus trichocarpa*	Aa_trans domain-containing protein
A0A068TQU2	7.40E-06	1	*Coffea canephora*	Alpha-mannosidase
A0A068V6G0	1.30E-05	1	*Coffea canephora*	Carboxypeptidase
A0A068TNE2	1.40E-05	1	*Coffea canephora*	Fn3_like domain-containing protein
A0A0K9RCY6	3.70E-05	1	*Spinacia oleracea*	Protein kinase domain-containing protein
A0A2G9I2W6	5.10E-05	1	*Lachancea mirantina*	Tetrahydroberberine oxidase

After the Biopep analysis of peptides found in CS extracts, several bioactivities have been highlighted (Figure S1). Among these biological activities, a pivotal role could be

played by peptides that contain sequences with angiotensin-converting-enzyme (ACE) and dipeptidyl peptidase IV (DPP IV) inhibitory activities. Although in a minor part, the peptides found in the extracts could also be precursors of antioxidant sequences like LKP and alpha-glucosidase inhibitors peptides such as VE and PP.

Table 4. Peptides found in the aqueous extract of coffee silverskin protein isolate (SPI).

Acc #	Peptide	E-value	Species	Protein Name
A0A068TNE2	DMHMRPDPATGYPGR	1.40E-05	COFCA	Fn3_like domain-containing protein
A0A068TQU2	QHHDAVTGTEQQHVANDYAK	7.40E-06	COFCA	Alpha-mannosidase
A0A068UEN7	IFYDENAQPVR	5.70E-10	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068UEN7	IFYDENAQPVRVK	5.70E-10	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068UEN7	DFFGKEYR	2.20E-09	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068UJ50	MGSGHFAEEGFR	1.40E-11	COFCA	Neprosin
A0A068UJ50	MGSGHFAEEGFRGA	1.40E-11	COFCA	Neprosin
A0A068UJ50	QMGSGHFAEEGFR	1.40E-11	COFCA	Neprosin
A0A068UJ50	QMGSGHFAEEGFRGA	1.40E-11	COFCA	Neprosin
A0A068UJ50	TQMGSGHFAEEGFRGA	1.40E-11	COFCA	Neprosin
A0A068UJ50	QMGSGHFAEEGFRGA	1.80E-08	COFCA	Neprosin
A0A068UJ50	TQMGSGHFAEEGFR	1.80E-08	COFCA	Neprosin
A0A068UNA6	DRPGLPFDEVGHGTH	5.50E-12	COFCA	Serine protease
A0A068UNA6	DRPGLPFDEVGHGTHTA	5.50E-12	COFCA	Serine protease
A0A068UNL8	DLLGPKPGQPLDEIGHGTH	9.60E-15	COFCA	Serine protease
A0A068UNL8	DLLGPKPGQPLDEIGHGTHTA	9.60E-15	COFCA	Serine protease
A0A068UNL8	DVFATGAGHVNPPR	9.60E-15	COFCA	Serine protease
A0A068UNL8	GAGHVNPPRAID	9.60E-15	COFCA	Serine protease
A0A068UNL8	SFSSRGPNNASPGILKPD	9.60E-15	COFCA	Serine protease
A0A068UNL8	STAAGNFVEGANVMR	9.60E-15	COFCA	Serine protease
A0A068UNL8	AGNFVEGANVMR	1.30E-12	COFCA	Serine protease
A0A068UNL8	ATGAGHVNPPRAID	1.30E-12	COFCA	Serine protease
A0A068UNL8	GVEIDVQPRVL	1.30E-12	COFCA	Serine protease

A0A068UNL8	NASPGILKPD	1.30E-12	COFCA	Serine protease
A0A068UNL8	SGITPGHPSFS	1.30E-12	COFCA	Serine protease
A0A068UNL8	SGITPGHPSFSD	1.30E-12	COFCA	Serine protease
A0A068UNL8	SRGPNNASPGILKPD	1.30E-12	COFCA	Serine protease
A0A068UNL8	SSRGPNNASPGILKPD	1.30E-12	COFCA	Serine protease
A0A068UNL8	YVDIDKIQGVEIDVQPR	1.30E-12	COFCA	Serine protease
A0A068V6G0	GAGHEVPSYRPD	1.30E-05	COFCA	Carboxypeptidase
A0A068V8L2	LGGESSDRPF	6.70E-06	COFCA	Glycoside_hydrolase_SF
A0A068V8L2	TGVVDQVRVE	6.70E-06	COFCA	Glycoside_hydrolase_SF
A0A068VNV4	AGDAPSPGAGSIEAIPHIPIHR	9.40E-11	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068VNV4	QDQSSALYNQNR	9.40E-11	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068VNV4	APSPGAGSIEAIPHIPIHR	3.40E-09	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068VNV4	DQSSALYNQNR	3.40E-09	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A068VNV4	EYRAGDAPSPGAGSIE	3.40E-09	COFCA	Tyrosinase_Cu-bd domain-containing protein
A0A0K9RCY6	HSNKLGDEIM	3.70E-05	SPIOL	Protein kinase domain-containing protein
A0A218WZL2	LFYDENAQPVR	2.20E-09	PUNGR	Polyphenol oxidase, chloroplastic-like
A0A218WZL2	LFYDENAQPVRVK	2.20E-09	PUNGR	Polyphenol oxidase, chloroplastic-like
A0A2G9I2W6	VGLVFNSYGGIM	5.10E-05	9LAMI	Tetrahydroberberine oxidase
B9N1V7	SKAGKSASYGGLM	6.10E-07	POPTR	Aa_trans domain-containing protein
M0SIL8	MGSGHFAEEGFGRA	1.40E-11	MUSAM	Neprosin
M0SIL8	QMGSGHFAEEGFGRA	1.40E-11	MUSAM	Neprosin
M0SIL8	TQMGSGHFAEEGFGRA	1.40E-11	MUSAM	Neprosin

This evidence may confirm the application of CS extract as supplementation to conventional drugs during diabetes treatments. Type 2 diabetes (T2D) is generally caused by a combination of insulin resistance and beta-cell failure that leads to elevated blood glucose levels (Veelen et al., 2021). A high blood sugar level can cause macrovascular complications (coronary artery disease, peripheral artery disease, and stroke), as well as microvascular complications (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy). In our body, incretins stimulate beta-cell growth, differentiation, and survival in addition to their

well-documented, potent insulinotropic effects. Dipeptidyl peptidase IV rapidly antagonistically inactivates these hormones in vivo, thus limiting their therapeutic potential. In humans and animal models of type 2 diabetes, inhibition of DPP IV enhanced circulating incretin levels, showing promising results for improving glucose tolerance, insulin sensitivity, and adiponectin function (Pospisilik et al., 2003).

CS contains bioactive compounds such as chlorogenic acid, caffeine, and melanoidins that are metabolized and interact with the cells involved in the pathogenesis of diabetes and its complications (Iriondo-DeHond et al., 2020a). It is well-known the association between coffee and coffee derived extracts consumption and reduction of blood glucose levels and beneficial effect on the glucose uptake by tissues and well-being of pancreas beta cells (Fernandez-Gomez et al., 2015, 2016; Chen et al., 2015; Abunasef et al., 2014). Our *in-silico* finding of ACE inhibitors, antioxidant and DPP IV inhibitor peptides are promising and may add information to what is already known about the effect of CS extracts on the treatment of diabetes and its side effects. However, the effect on diabetic patients of the biopeptides precursors found in CS should be confirmed by the development of synthetic peptides, and the release of the effective bioactive peptides after in-vitro digestion of the extracts should be further validated.

3.3. Morphological-structural characterization

Finally, the last objective of this work was to carry out analyses for a morphological and structural characterization of CS, to evaluate its possible applications also in industrial fields. To this end, analyses were performed with Fourier Transform Infrared (FT-IR) spectroscopy,

CP/MAS 13C NMR solid-state spectroscopy, thermogravimetric analysis (TGA), Scanning Electron Microscopy (SEM) analysis and volumetric nitrogen adsorption analysis.

Fourier Transform Infrared (FT-IR) spectroscopy was used to identify the functional groups present in the CS. The FT-IR spectra of CS compared to pure cellulose are shown in Figure 3. The predominant peaks observed at 1000 cm^{-1}, in the 1200 – 1450 cm^{-1} region and at 3350 cm^{-1}, marked the presence of cellulose and lignin and hemicellulose, as revealed by the strong absorption bands encompassing the aromatic ring and carbonyl vibration regions (1500 – 1750 cm^{-1}). Also, the narrow signals observed at 2800 – 2900 cm^{-1} (C-H stretching region) are usually present in lignin and hemicellulose spectra (Horikawa et al., 2019). Finally, the complex shape of the carbonyl absorption band suggests the presence of different carbonyl-containing species.

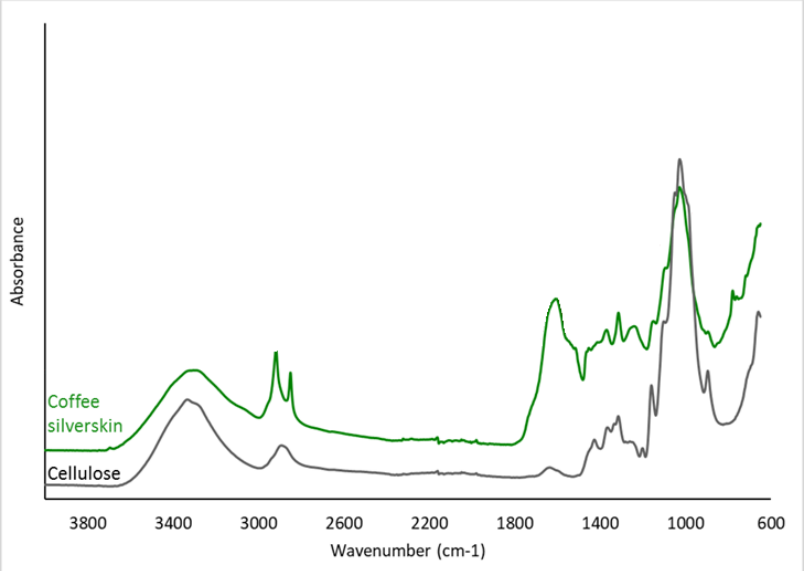

Figure 3. Fourier Transform Infrared (FT-IR) spectroscopy of coffee silverskin (green) and a pure cellulose sample (black) reported as a reference

Cross-Polarization/Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance - (CP/MAS 13C NMR) is widely used to study the chemical composition of lignocellulosic

biomass and analyze changes that occur during chemical/thermal treatments (Kostryukov et al., 2021). The spectrum obtained on CS supports and complements the compositional analyses reported in previous sections. Indeed, the main peaks observed can be identified as cellulose, hemicellulose, and lignin, which are the main structural components of the integument (Figure 4). Other signals observed in the NMR spectrum can be related to other essential components of CS. In the high field range (10-40 ppm), signals related to aliphatic carbons are detected, which could be attributed to protein fractions. At low field (165-185 ppm), a complex and wide signal attributed to carbonyl groups can be ascribed to the amide carbonyls of proteins and oxidized groups of lignin and hemicellulose. Lignocellulosic materials are widely recognized as interesting biosourced reinforcements for polymer-based composites (Ortega et al., 2021; Avolio et al., 2015) for their excellent mechanical properties and wide availability. The presence in such materials of polyphenol aromatics like lignin and its derivatives is often associated with antioxidant properties that can be exploited in composites to confer functional properties of high applicative interest. Natural antioxidants can have beneficial effects on the stability of polymers during processing and/or reprocessing, avoiding the use of synthetic stabilizers (Kirschweng et al., 2017) and favoring recyclability. On the other hand, if composite films are produced for packaging applications, the migration of antioxidant molecules at the film surface can be promoted, extending the oxidative stability of the contained foods. This functional property can limit the need for preservatives and extend the product's shelf life (Zhang et al., 2021).

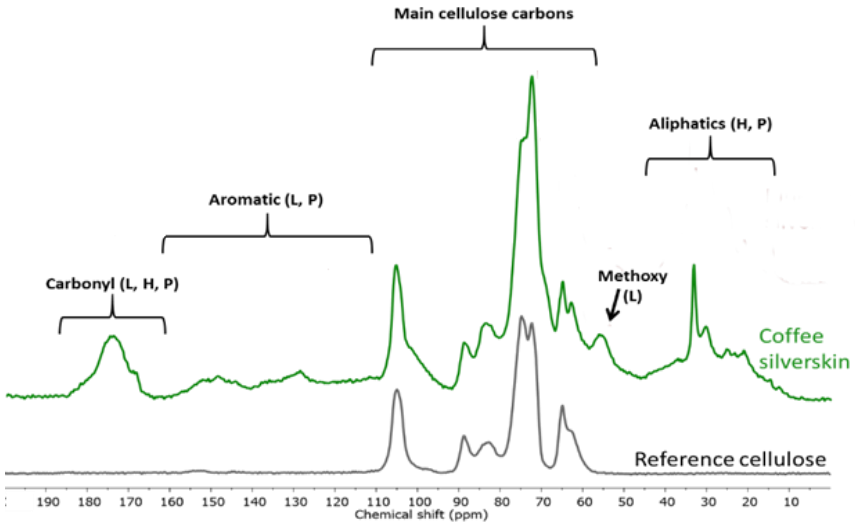

Figure 4. Cross-Polarization/Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CP/MAS ^{13}C NMR) spectra of coffee silverskin (green) and a pure cellulose sample (black) reported as a reference. A qualitative attribution of the main signals observed is reported, indicating cellulose, lignin (L), hemicellulose (H), proteins (P).

The thermogravimetric (TG) analysis allows determining the thermal stability of materials. This analytical technique is based on evaluating the mass loss at controlled temperatures over time in conditions of a controlled atmosphere (Górska et al., 2020). TG can provide helpful information on the thermal decomposition mechanisms of plant biomass due to the decomposition characteristics at given temperatures in an inert atmosphere of the lignocellulosic fraction, from 250 °C for hemicellulose up to 500 °C for lignin (Carrier et al., 2011). The thermogravimetry–differential thermal analysis (TG–DTG) curve carried out on CS sample, as shown in Figure 5, is characterized by three defined mass loss steps. The first one started at 80°C and was attributed to the evaporation of surface-bound water (natural humidity), presenting a low weight loss, around 4%. The second degradation step, between 200 and 500 °C, could be attributed to the overlapping of the main degradation processes (dehydration, decarboxylation, depolymerization and decomposition) of the other cellulose,

hemicellulose and lignin fractions. The maximum of the DTG curve is recorded at 320°C and indicates the point of the most significant rate of change on the weight loss curve. The weight loss associated with the second degradation step was 61 wt%. Finally, a slower degradation stage until 800 °C was observed with a further weight loss of 10%. This CS thermal characterization is consistent with that performed by Górska et al., 2020, in which the three steps of the curve represented had similar percentages of mass losses, the only difference being that we went up to 800°C with a residual char equal to 25 wt% while they recorded 31.6% undecomposed CS material at 700°C. In addition, a study conducted by del Pozo et al., 2020 characterized the bio-oil and residual biochar resulting from the intermediate pyrolysis process at 280°C. They noted that at this temperature from the bio-oil was obtained the highest phenolic content of 6.09 mg of gallic acid equivalents/g while the biochar presented significant caloric values (22 MJ/kg). This opens an interesting prospect of CS use for a biorefinery approach.

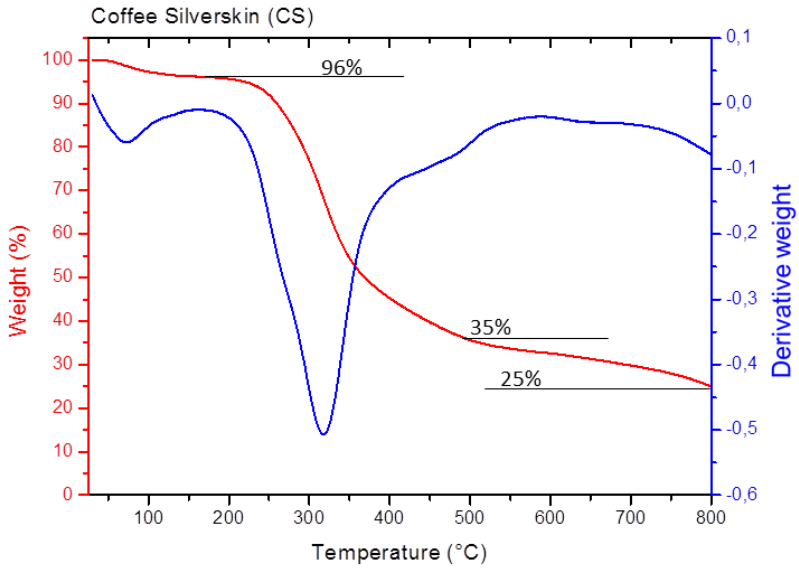

Figure 5. Thermogravimetric and derivative curves of coffee silverskin

Morphological analysis of CS performed by SEM, as illustrated in Figure 6a, shows a compact and wrinkled surface, suggesting that the fibrous cellulose structure is embedded in a matrix of less structured hemicellulose/lignin components. At higher magnification, the presence of the cellulosic fibers is clearly visible, with thickness of about 1.3 – 1.5 µm. Furthermore, almost spherical particles about 0.5 – 5 µm in size appear deposited on CS sheets (Figure 6b), that could be ascribable to the lignin and/or protein fraction.

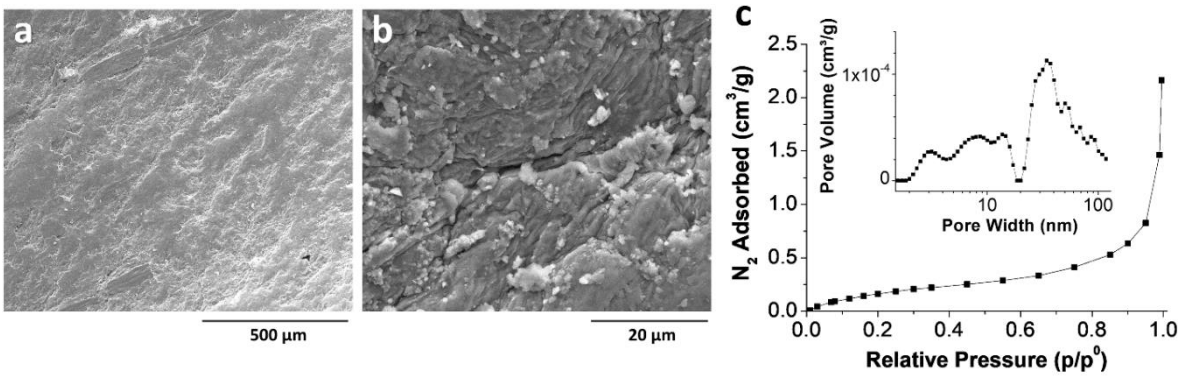

Figure 6. Scanning Electron Microscopy (SEM) images (a, b), N_2 adsorption isotherm and pore size distribution (c) of CS.

The measurement of gas adsorption isotherms is a well-established technique in the characterization of porous materials (Sotomayor et al., 2018), and the nitrogen adsorption (NA) analysis at 77 K is a standard tool for this purpose (Reichenauer and Scherer, 2000). The nitrogen adsorption isotherm of CS is a type III isotherm with slightly pronounced adsorption at low pressures, indicating a porous structure characterized by large mesopores and macropores (Figure 4c). Indeed, NLDFT pore size distribution (inset in Figure 6c) reveals that CS porosity extends from 2 nm to about 100 nm, with a major distribution peak at about 35 nm. Overall, CS shows limited porosity, with a total pore volume of about 0.002 cm^3/g and a BET SSA of about 0.77 ± 0.01 m^2/g, as expected for this kind of natural resource in its native state (Ballesteros et al, 2014). However, CS porosity can constitute the basis for the

realization of a micro/mesoporous material through proper thermochemical treatments aimed at enhancing the CS adsorption behavior (del Pozo et al., 2021).

Summarizing, morphological and structural analyses could find practical utility as they represent an essential knowledge basis for the use of CS as a filler for creating bio-composites. An interesting study by Hejna et al., 2021 showed that the addition of even small amounts of silverskin increases the thermo-oxidative resistance of high-density polyethylene (HDPE) composites, hypothesizing a very beneficial impact on the development of new wood polymer composites (WPC) and natural fiber composites (NFC).

4. Conclusions

An accurate characterization of a waste material is essential for a complete evaluation of its reuse potential. The circular economy, characterized by the "zero waste" paradigm, is focusing on the development of a new idea of consumption that is increasingly green and eco-sustainable. The possibility of reusing waste materials deriving from the agri-food sector could be considered as an economic and environmental solution deriving from the inappropriate disposal of these by-products and moreover provide products with high benefits for human health and with lower environmental impact. As our study showed, by-products such as CS could be used for a variety of purposes, from food to nutraceuticals, not excluding possible uses as fillers in composites in the production of materials. Further studies need to be conducted to better understand the possible uses of this byproduct in order to reduce processing costs and encourage the coffee chain to manage processing waste in a more environmentally friendly manner.

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Coffee Silverskin: Chemical and Biological Risk Assessment and Health Profile for Its Potential Use in Functional Foods

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Abstract

The coffee supply chain is characterized by a complex network with many critical and unsustainable points producing a huge amount of waste products. Among these, coffee silverskin (CS), the only by-product of the coffee roasting phase, has an interesting chemical profile that suggests potential use as a food ingredient. However, few data on its safety are available. For this reason, the purpose of the study was to assess the occurrence of chemical and biological contaminants in CS, and the resulting risk due to its potential consumption.

Essential, toxic, and rare earth elements, polycyclic aromatic hydrocarbons (PAHs), process contaminants, ochratoxin A (OTA), and pesticides residues were analyzed in three classes of samples (*Coffea arabica* CS, *Coffea robusta* CS, and their blend). Furthermore, total mesophilic bacteria count (TMBC) at 30 °C, Enterobacteriaceae, yeasts, and molds was evaluated. The risk assessment was based upon the hazard index (HI) and lifetime cancer risk (LTCR).

In all varieties and blends, rare earth elements, pesticides, process contaminants, OTA, and PAHs were not detected except for chrysene, phenanthrene, and fluoranthene, which were reported at low concentrations only in the *arabica* CS sample. Among essential and toxic elements, As was usually the most representative in all samples. Microorganisms reported a low load, although *arabica* and *robusta* CS showed lower contamination than mixed CS. Instead, the risk assessment based on the potential consumption of CS as a food ingredient did not show either non-carcinogenic or carcinogenic risk. Overall, this study provides adequate evidence to support the safety of this by-product for its potential use in functional foods.

Keywords: Silverskin; Coffee by-products; Risk assessment; Agri-food waste; Dietary exposure.

1. Introduction

Coffee is one of the most consumed beverages in the world and is the second most traded product, after petroleum, with a market volume of about $15 billion (Lanfranchi et al., 2016). The primary coffee production comes from South America, particularly Brazil, which has recorded an 18.5% increase in production in 2019–2020 (ICO, 2021). *Coffea arabica* is the most dominant variety in the coffee market, with 175,347 thousand 60 kg bags in 2020, compared to 70,086 thousand 60 kg bags for *Coffea canephora* var. *robusta* (ICO, 2021). Due to the enormous amount of coffee produced, marketed, and consumed, the coffee supply chain faces an environmental pollution burden, also related to the disposal of by-products.

Coffee cherry undergoes numerous processing steps that transform the raw fruit into liquid coffee. These processing steps are made to separate the outer layers that cover the green coffee bean. The structure of the coffee bean is composed as follows: husk (exocarp), pulp (mesocarp), parchment (endocarp), silverskin (integument), and finally, the two beans that constitute the final product of the processing chain from which the beverage is obtained (Alves et al., 2017). Briefly, reviewing the processing steps, the coffee cherry, after being harvested, with a first discharge of the unsuitable cherries, is subjected to a wet or dry process (Oliveira et al., 2021). The wet method involves removing the pulp from the bean by fermentation.

On the other hand, the dry method involves drying and hulling the coffee cherry and collecting husk, pulp, and parchment, which are disposed of as waste (Alves et al., 2017). At this point, the green coffee beans, obtained from the previous processing steps, are packaged and shipped to consumer countries where roasting occurs. The roasting phase is a crucial part of coffee processing; it influences the organoleptic characteristics of the final beverage (López-Galilea et al., 2006). The coffee roasting phase includes blending different coffee

varieties, with a roasting temperature of about 200 °C for less than 20 min, with substantial variability in time and temperature depending on the roasting system adopted (Esposito et al., 2020).

The only by-product of this step is the coffee silverskin (CS), a thin integument obtained by detachment from the green bean due to the high temperatures reached during roasting. Finally, the roasted coffee bean is ready to be used to obtain the well-known beverage. The CS has a low mass (1% to 2% of the whole bean's weight), but the total amount of this by-product is considerable in relation to the coffee consumed each year. Some studies on its potential reuse in cosmetics, nutraceuticals, and industry (Bessada et al., 2018; Peixoto et al., 2022; Ghazvini et al., 2022). Moreover, given its chemical-physical profile related to the good amount of protein (19%), dietary fiber (30–70%), phenolic compounds, and melanoidins, CS is earning much interest for possible use as food or food ingredient (Mesías & Delgado-Andrade, 2017; Bertolino et al., 2019; Tores de la Cruz et al., 2019; Iriondo-DeHond et al., 2019b; Martuscelli et al., 2021b).

To be included in the list of novel foods, it must undergo the authorization procedure set by Regulation (EU) No. 2015/2283. At present, coffee silverskin is evaluated as a novel food requiring pre-market approval (Regulation (EU) 2015/2283). The European Commission is responsible for authorizing novel foods and, as part of the procedure, may ask the European Food Safety Authority (EFSA) to conduct a scientific risk assessment to establish their safety (Klingel et al., 2020). In order to use it as a novel food, it is necessary to evaluate the absence of chemical and microbiological contaminants in this product, thus proceeding to a risk assessment. To this end, the present study aims to provide a comprehensive characterization investigating the occurrence of heavy metals, pesticides, rare earth elements (REEs), polycyclic aromatic hydrocarbons (PAHs), and biological contaminants. Since chemical

features of coffee could differ among varieties (Liu et al., 2022), the silverskin obtained from the roasting of *Coffea arabica*, *Coffea robusta*, and a blend of both were studied. Ultimately, a risk assessment based on deterministic simulation was carried out considering the potential consumption of CS as a food ingredient.

2. Materials and Methods

2.1. Coffee Silverskin Sample

The arabica and robusta silverskin were obtained by roasting *Coffea arabica* and *Coffea robusta* green beans. The roasting was assessed in the laboratory using a Probatino rotary drum roaster (Probat, Emmerich am Rhein, Germany), equipped with a display to monitor time and temperature. The controlled curves of roasting temperatures were set out according to Esposito et al., 2020. The mixed silverskin was provided by two coffee industries of the Campania region (Italy), obtained by blending roasted green beans of *Coffea arabica* and *Coffea robusta* (in unspecified ratio). Coffee varieties are usually blended by the companies during the roasting process, then the CS is recovered with suction cyclones and collected (Lachenmeier et al., 2021). All the CS samples were stored in the dark at 20 °C and analyzed within three days.

2.2. Chemical Analysis

2.2.1. Essential, Toxic, and Rare Earth Elements

All solution preparation and sample dilution for multi-elemental analysis were made with high-purity water (resistivity of 18.2 MΩ cm) obtained from a Milli-Q unit (Millipore, Burlington, Massachusetts, USA). Nitric acid (HNO_3, 69% *v/v* Ultratrace® ppb-trace analysis

grade) and hydrofluoric acid (HF, 48% *v/v* Ultratrace® ppb-trace analysis grade) were provided by Scharlau (Barcelona, Spain). Multi-component certified solution of 30 elements (ultrapure grade for ICP) was provided by Ultrascientific (Bologna, Italy). Boric acid (99.97% trace metals basis) and multi-component certified solution of 16 rare earth elements REE (50 mg/L each, ultrapure grade for ICP, TraceCERT®) were purchased from Merck (Darmstadt, Germany). All glassware and plastic containers used for the preparation of samples and standards were tested and found free of analyzed elements.

Multi-elemental analysis was carried out by the Inductively Coupled Plasma—Mass Spectrometry (ICP-MS) and Microwave Plasma-Atomic Emission Spectrometry (MP-AES) after digestion.

An aliquot of 250 ± 1 mg of each sample was digested with 10 mL of ultrapure nitric acid (HNO_3, 69% *v/v* Ultratrace® ppb-trace analysis grade) and 1 mL of hydrofluoric acid (HF, 48% *v/v* Ultratrace® ppb-trace analysis grade) in PP test tubes. The latter were placed in a water bath, preheated to 80 °C and proceeded to digestion for 2 h. After this time, 400 mg of boric acid were added to the mixture, and digestion continued for 1 h. Samples were brought to a final volume of 20 mL with HNO_3 solution (2%, *v/v*) for the subsequent elemental analysis.

In this case, 19 elements (As, Sb, Ba, Be, B, Cd, Co, Cr, Fe, Mn, Hg, Ni, Pb, Cu, Se, Sn, Tl, V, Zn) and 15 REEs (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) were analyzed by Aurora M90 ICP-MS instruments (Bruker, Bremen, Germany). Na, K, Mg and Ca were analyzed by 4210 MP-AES instruments (Agilent, Santa Clara, CA, USA). Calibration curves were obtained in the range from 1 to 100 µg/L for ICP-MS and in the range from 0.1 to 100 mg/L for MP-AES for each analyzed element from certified standard solutions. A blank for reagents and a blank for the digestion method were performed to verify

contamination of all materials, and calibration was verified in the instrumental sequence with two control standards for every 20 samples.

The limit of quantification (LOQ) was calculated by the method of blanks variability for each investigated element, and they are included in the range 0.05–1 mg/kg in the final sample for ICP-MS analysis and 10 mg/Kg in the final sample for metals detected with MP-AES.

2.2.2. Polycyclic Aromatic Hydrocarbons (PAHs)

Sample preparation and solutions for PAHs analysis were made with acetone (≥ 99.8%, GC grade) and n-hexane (≥95%, GC grade). Multi-component certified solution of 15 PAHs (2000 µg/mL each) and deuterated internal standard mixture solution (100 µg/mL each) were provided by Ultrascientific (Bologna, Italy). All glassware was tested and found free of PAHs.

An aliquot of 1.000 g ± 1 mg of each sample was extracted with 25 mL acetone/n-hexane 1:1 *v/v* using an ultrasonic bath (Branson Ultrasonic Corporation, Brookfield, Connecticut, USA) for three 30-min cycles in closed glass vials. After this time, the extract was concentrated to a final volume of 1 mL using automatic concentrator MultiVap 8 (Labtech, Bergamo, Italy), and the purification procedure was unnecessary for the subsequent GC-MS analysis. The extract was injected into a gas chromatograph coupled with a mass spectrometer (GC 2010 Plus, MS-TQ8030, Shimadzu, Kyoto, Japan) for the determination of 15 PAHs (Arienzo et al., 2017).

Calibration curves were obtained in the range from 1 to 100 µg/L by calculating the area ratio of the target ion to the respective internal standard with five standard solutions obtained from certified standard solutions. A blank for reagents and a blank for the digestion method

were performed to verify contamination of all materials, and calibration was verified in the instrumental sequence with a control standard for every ten samples.

The limit of quantification (LOQ) was calculated by the method of blanks variability and was equal to 0.05 mg/kg in the final sample.

2.2.3. Acrylamide (AA) and Furan

The acrylamide (AA) was analyzed following the Fernandes and Soares (2007) method with some modifications. A solid-phase extraction dispersed with C18 sorbent packed in polypropylene columns was performed by taking an aliquot of 0.5 g of homogenized sample. Then, the aqueous eluate was derivatized after bromination and subsequent GC-MS reading and quantification of the derivative. The limit of quantification (LOQ) was 0.02 mg/kg.

The furan was analyzed with headspace technique following the Park et al., 2021 method. An aliquot of 5 g of sample and 5 mL of HPLC water were added to a headspace vial (20-mL) and carefully sealed. Then d4-furan solution (internal standard) was added to the vial, homogenized and placed on ice. Furan was extracted from the samples by an automated solid-phase micro-extraction (SPME) agitating the sample at 300 rpm, 50 °C. The fiber (carboxen/polydimethylsiloxane) was exposed to the headspace of vial samples for 20 min at 25 mm depth. Then, the fiber was thermally desorbed in the injection port of a gas chromatograph at 40 mm depth for 5 min. The limit of quantification (LOQ) was 0.1 mg/kg.

2.2.4. Ochratoxin A (OTA)

The analysis of ochratoxin A (OTA) was performed following the official method EN 1413:2003 with some modifications. 10 g of sample were homogenized with methanol:water solution (70:30 *v*/*v*), and it was diluted with a phosphate buffer solution prepared by dissolving 160 g of NaCl, 4.0 g of KCl, and 36.0 g of sodium hydrogen phosphate dihydrate

(Na_2HPO_4-$2H_2O$) in 1800 mL of water, adjusting the pH of the solution to 7.4 with 0.1 M HCl or with 0.1 M NaOH. Then, purification was performed by immunoaffinity chromatography onto columns containing a gel on which anti-OTA antibodies are immobilized. After, mycotoxin was eluted with pure methanol and quantified by HPLC with a reversed-phase C18 column and a fluorimetric spectrum detector. The limit of quantification (LOQ) was 0.1 mg/kg.

2.2.5. Pesticides Residues

Dispersive SPE—Modular QuEChERS (Quick Easy Cheap Effective Rugged Safe) method was used for acetonitrile extraction and purification of pesticide residues CS according to European standards EN 15662:2018. The compound investigated are listed in Supplementary Table S1. Pesticide residues were analyzed in duplicate by a multimethod procedure using GC-MS/MS and LC-MS/MS with a LOQ (limit of quantification) of 0.01 mg/kg.

2.3. Microbiological Analysis

Total Mesophilic Aerobic Bacteria (TMAB), Lactic Acid Bacteria (LAB), Enterobacteriaceae, yeasts and molds and Salmonellae were detected to evaluate the microbiological contamination of coffee silverskin. CS was serially diluted in Quarter-Strength Ringer's solution (1:10 *w*/*v*) (Basingstoke, UK). An inoculum of 0.1 and 1 mL was then transferred from dilutions into (pour plate method) and onto (spread plate method) appropriate medium. TMAB were counted on Plate Count Agar (PCA) after 48 h of incubation at 30 °C. LABs were counted on deMan Rogosa and Sharp (MRS) Agar after 48 h of incubation at 30 °C. Enterobacteriaceae were counted on Violet Red Bile Glucose Agar

(VRBGA) after 48 h of incubation at 37 °C. Yeasts and Molds were counted on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar after 48 h of incubation at 28 °C. Salmonellae were detected according to UNI EN ISO 6579-1: 2020, which involves an initial pre-enrichment step in Buffered Peptone Water at a ratio of 1:10. After incubation at 34–38 °C for 18 h, 0.1 mL was transferred into 10 mL of Rappaport-Vassiliadiscon Soy Broth (RVS) and reincubated at 41.5 °C for 24 h, while 1 mL was used to inoculate 10 mL of Muller-Kauffmann to Tetrationate-novobiocin broth (MKTTn) reincubated subsequently at 37 °C for 24 h. For the next isolation step, Xylose Lysine Deoxycholate (XLD) agar and Brilliance Salmonella Agar Base (BSA) agar plates were seeded by streaking from RVS and MKTTn cultures for each culture broth, which was incubated at 37 °C for 24 h.

2.4. Risk Assessment

The risk due to potential consumption of coffee silverskin was assessed through hazard-quotient (HQ) and lifetime cancer risk (LTCR). HQ based on estimated daily intake (EDI) was calculated through Equations (1) and (2). An HQ> 1, indicates a likely high risk as far as non-carcinogenic adverse effects are concerned.

$$EDI_k = \frac{C_k \; x \; IR}{BW} \quad (1)$$

$$HQ_k = \frac{EDI_k}{TDI_k} \quad (2)$$

Ck: Concentration of contaminant k detected in silverskin (mg/kg).

IR: Intake Rate of silverskin (kg/day).

BW: Body Weight of 70 kg.

TDI: tolerable daily intake.

The risk derived from cumulative exposure was calculated through the Hazard Index (Equation (3)). HI > 1 indicates a likely high no-carcinogenic risk due to exposure to multiple contaminants.

$$HQ_k = \frac{EDI_k}{TDI_k} \quad (3)$$

LTCR was estimated for carcinogenic contaminants based on their slope factor (SF), as shown in equation 4. Values above 1×10^{-4} are considered unacceptable for the risk of developing cancer over a human lifetime. Whereas values between 1×10^{-6} and 1×10^{-4} are considered an acceptable range for risk according to USEPA, 2001.

$$LTCR = (EDI \times EF \times TE \times SF)/AT \quad (4)$$

EF: Exposure Frequency to the contaminant (350 day/year).

TE: Total Exposure (70 year).

AT: Average Lifetime time for non-carcinogenic risk (TE × 365 day/year).

SF: Slope Factor $(mg/kgbw/day)^{-1}$ related to each PAE (Table 1).

3. Results and Discussion

3.1. Chemical Contaminants

During coffee roasting, the high temperature produces a chemical change in coffee beans composition and the formation of new organic compounds as a result of the Maillard reaction and pyrolysis of nonvolatile compounds (Toci & Farah, 2014; Esposito et al., 2020). These include toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) and acrylamide (AA) (Pissinatti et al., 2015; Cagliero et al., 2016). Our preliminary study Nolasco et al., (2022) evaluated the concentration of process contaminants such as acrylamide (AA),

furan, methyl furan and ochratoxin A (OTA) and some heavy metals and PAHs in CS for its multipurpose recycling applications. This study performed an extensive semiquantitative elemental screening of the CS chemical profile. Several classes of contaminants, such as heavy metals, PAHs, process contaminants, OTA, and pesticides, were investigated in *Coffea arabica*, *robusta*, and mixed silverskin. The summary data are listed in Tables 1–5.

The contents of the elements analyzed are mostly comparable to each other in the three respective CS samples, except for barium (Ba), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni). In detail, *robusta* CS has a higher Fe content (442 mg/kg) than *arabica* CS (179 mg/kg), while mixed CS has an intermediate value (319 mg/kg). A similar trend occurs for Cu content, with *robusta* CS (132 mg/kg), mixed CS (106 mg/kg), and *arabica* CS (35.5 mg/kg). In contrast, *arabica* CS shows higher values for Ba and Mn, 60.6 mg/kg and 53.1 mg/kg, respectively. The mixed CS reports for almost all elements values intermediate to the two varieties, except for Ni (2.41 mg/kg), Co (0.68 mg/kg) and V (0.76 mg/kg) with higher values and As (0.21 mg/kg) and Be (0.05 mg/kg) with lower values than the two coffee varieties.

Table 1. Concentrations (mg/kg) of essential, toxic, and rare earth elements in three classes of samples (n = 10) of coffee silverskin (CS).

Elements	***Robusta* (mg/kg)**	**Mixed (mg/kg)**	***Arabica* (mg/kg)**
Antimony Sb	<LOQ	<LOQ	<LOQ
Arsenic As	0.29	0.21	0.27
Barium Ba	28.2	49.4	60.6
Beryllium Be	0.06	0.05	0.06
Boron B	31.3	32.4	33.1
Cadmium Cd	<LOQ	<LOQ	0.15
Cobalt Co	0.45	0.68	0.36
Chromium Cr	0.59	0.48	0.26
Iron Fe	442	319	179
Manganese Mn	20.8	24.6	53.1
Mercury Hg	0.06	0.06	0.07
Nickel Ni	1.08	2.41	0.91
Lead Pb	0.36	<LOQ	<LOQ
Copper Cu	132	106	35.5
Selenium Se	<LOQ	<LOQ	<LOQ
Tin Sn	<LOQ	<LOQ	<LOQ
Thallium Tl	<LOQ	<LOQ	<LOQ
Vanadium V	0.58	0.76	<LOQ
Zinc Zn	17.9	15.8	11.2
Yttrium Y	<LOQ	<LOQ	<LOQ
Lanthanum La	<LOQ	<LOQ	0.07
Cerium Ce	<LOQ	<LOQ	0.07
Praseodymium Pr	<LOQ	<LOQ	<LOQ
Neodymium Nd	<LOQ	<LOQ	<LOQ
Samarium Sm	<LOQ	<LOQ	<LOQ
Europium Eu	<LOQ	<LOQ	<LOQ
Gadolinium Gd	<LOQ	<LOQ	<LOQ
Terbium Tb	<LOQ	<LOQ	<LOQ
Dysprosium Dy	<LOQ	<LOQ	<LOQ
Holmium Ho	<LOQ	<LOQ	<LOQ
Erbium Er	<LOQ	<LOQ	<LOQ
Thulium Tm	<LOQ	<LOQ	<LOQ
Ytterbium Yb	<LOQ	<LOQ	<LOQ
Lutetium Lu	<LOQ	<LOQ	<LOQ

Table 2. Concentrations (mg/kg) of polycyclic aromatic hydrocarbons (PAHs) in three classes of samples (n = 10) of coffee silverskin (CS).

Polycyclic Aromatic Hydrocarbons	*Robusta* (mg/kg)	Mixed (mg/kg)	*Arabica* (mg/kg)
Naphthalene	<LOQ	<LOQ	<LOQ
Acenaphtilene	<LOQ	<LOQ	<LOQ
Acenaphthene	<LOQ	<LOQ	<LOQ
Fluorene	<LOQ	<LOQ	<LOQ
Anthracene	<LOQ	<LOQ	<LOQ
Phenanthrene	<LOQ	<LOQ	0.07
Fluoranthene	<LOQ	<LOQ	0.18
Pyrene	<LOQ	<LOQ	<LOQ
Chrysene	<LOQ	<LOQ	0.06
Benzo(b)fluoranthene	<LOQ	<LOQ	<LOQ
Benzo(k)fluoranthene	<LOQ	<LOQ	<LOQ
Benzo(a)pyrene	<LOQ	<LOQ	<LOQ
Indeno[1,2,3-cd]pyrene	<LOQ	<LOQ	<LOQ
Dibenz[a,h]anthracene	<LOQ	<LOQ	<LOQ
Benzo[ghi]perylene	<LOQ	<LOQ	<LOQ

Table 3. Concentrations (mg/kg) of process contaminants in three classes of samples (n = 10) of coffee silverskin (CS).

Process Contaminants	*Robusta* (mg/kg)	Mixed(mg/kg)	*Arabica* (mg/kg)
Acrylamide (AA)	<LOQ	<LOQ	<LOQ
Furan	<LOQ	<LOQ	<LOQ
Methyl-furan	<LOQ	<LOQ	<LOQ

Table 4. Concentrations (mg/kg) of *mycotoxins* in three classes of samples (n = 10) of coffee silverskin (CS).

Mycotoxins	*Robusta* (mg/kg)	Mixed (mg/kg)	*Arabica* (mg/kg)
Ochratoxin A (OTA)	<LOQ	<LOQ	<LOQ

Table 5. Concentrations (mg/kg) of pesticides (showed in Supplementary Table S1) in three classes of samples (n = 10) of coffee silverskin (CS).

Pesticides	*Robusta* (mg/kg)	Mixed (mg/kg)	*Arabica* (mg/kg)
Supplementary Table S1	<0.01	<0.01	<0.01

From literature, Ballesteros et al. (2014), Zarrinbakhsh et al. (2016), and Martuscelli et al. (2021a) assessed the levels of some mineral elements in an unspecified mixture of *arabica* and *robusta* coffee silverskin. Comparing these studies with our mixed CS results, Zarrinbakhsh et al. (2016) reported higher values for iron (843.30 mg/kg), zinc (22.30 mg/kg), cobalt (21.39 mg/kg), and chromium (1.59 mg/kg), lower value for copper (63.30 mg/kg) while elements such as barium, boron, lead, selenium, cadmium, and tin were in line with ours. Instead, Zarrinbakhsh et al. (2016) reported higher values for iron (660 mg/kg), zinc (27 mg/kg) and chromium (1.8 mg/kg) and lower values for copper (30 mg/kg). Another study (Martuscelli et al., 2021a) reports higher values of chromium (5.55 mg/kg) while lower values of iron (212 mg/kg) and copper (72.15 mg/kg). Furthermore, from the analyses conducted for our previous study, the arsenic and copper content in the mixed CS was higher, while zinc and iron had lower values than in the current study (Nolasco et al., 2022). These differences are probably due to the different compositions of the coffee blends produced in the roasting companies, which result in a different chemical profile of the silverskin obtained. For example, according to the study Gottstein et al. (2021), a CS pellet consisting probably of 70% *Coffea arabica* and 30% *Coffea canephora*, i.e., the typical coffee blend commercially available in Germany, has a different chemical profile from our mixed CS and the other previously cited works. Once again, the study Gottstein et al. (2021) characterized *arabica* CS with higher values for many elements analyzed, particularly for manganese (145 mg/kg), barium (130 mg/kg) and copper (98 mg/kg) than our *arabica* CS, and *robusta* CS with higher values for barium (73 mg/kg), copper (185 mg/kg), nickel (2.3 mg/kg) and chromium (2.9 mg/kg) than our *robusta* CS. Another study Nzekoue et al. (2022), analyzed CS from roasting *Coffea arabica* green beans and reported higher values for iron (238 mg/kg) and zinc (31.9 mg/kg), while lower values are reported for manganese (46.7 mg/kg). As regards PAHs, their

occurrence in coffee silverskin is critical information for risk assessment purposes. They are dangerous ubiquitous organic pollutants, and some are classified as probable human carcinogens due to their toxic, carcinogenic and mutagenic nature (Haritash A. K., 2020).

Among the three types of CS analyzed, only *arabica* CS reported the occurrence of Phenanthrene (0.07 mg/kg), Fluoranthene (0.18 mg/kg), and Chrysene (0.06 mg/kg). However, from our results, most of the elements and PAHs in *arabica*, *robusta* and mixed silverskin samples were reported at concentrations <LOQ. Similarly, AA, furan, methyl-furan, and OTA were not detected consistent with our previous investigation (Nolasco et al., 2022).

Lastly, analyses were performed to determine the occurrence of pesticides. A study by Coltro et al. (2006) evaluated the life cycle assessment (LCA) of green coffee production in Brazil; the results showed that, in addition to a considerable amount of water, 900 kg of total fertilizer and 10 kg of pesticides are used to produce 1,000 kg of coffee. This information, related to the botanical differences between the two coffee varieties, could influence the presence of pesticides in coffee cherries. Indeed, the depth of the root system is different: the *Coffea arabica* roots penetrate deeper into the soil, while the *robusta* roots are highly concentrated near the soil surface (Murthy & Naidu, 2012). However, analysis of the multiple pesticides searched (Supplementary Table S1) revealed no outliers, and all values detected were at <LOQ concentrations.

3.2. Microbiological Contaminants

For a more comprehensive characterization of CS, the occurrence of some biological contaminants such as Enterobacteriaceae was assessed. These bacteria are biological markers of food safety that could indicate the occurrence of pathogens. A higher Enterobacteriaceae

microbial load (0.78 ± 0.16 Log/UFC) was reported in mixed CS. However, all samples reported no occurrence of *Salmonella spp*. Furthermore, yeasts and molds were isolated from mixed CS, reporting a load of 2 ± 0.21 Log/UFC. Instead, the TMBC was 5.45 ± 0.17 Log/UFC in mixed CS, whereas *robusta* and *arabica* CS reported values of 3.61 ± 0.18 Log/UFC and 3.73 ± 0.22 Log/UFC, respectively. However, it is plausible that the high temperature of the roasting process and the low moisture content of CS (4–7%) (Toschi et al., 2014) limits its microbial load and extends its shelf life (Martuscelli et al., 2021a). In addition, to date, some studies have evaluated a potential application of CS in the formulation of food products that could ensure healthiness after the baking process. In particular, Pourfarzad et al., (2013) and Gocmen et al., (2019) evaluated its applicability in bakery products, whereas an interesting study by Martuscelli et al., (2021b) considered the formulation of a chicken meat burger with CS that would increase the shelf life of the meat due to its antioxidant activities in addition to a fiber supplement and an excellent source of minerals such as calcium, potassium and others.

3.3. Risk Assessment

CS is a high-nutritional value product with a fiber content of about 30–70% (Narita and Inouye, 2014; Behrouzian et al. 2016, Iriondo-DeHond et al., 2019a). Hence, a content of 5–10 g could provide more than 3 g of fiber allowing the use of the nutritional claims of "source of fiber" in CS-based products according to Regulation (EC) No 1924/2006. Based on this consideration, a consumption of 5 g and 10 g as food ingredients was supposed for the risk assessment. The EDI of each contaminant was calculated and compared with the corresponding threshold value expressed as TDI (Supplementary Table S2). The EDI was not calculated for compounds with concentrations <LOQ. The ratio between EDI and TDI, known

as HQ, was listed in Supplementary Table S2. The HQ ranged from 1.42×10^{-4} to 6.92×10^{-2}, from 1.15×10^{-4} to 5.45×10^{-2}, and from 6.12×10^{-5} to 6.50×10^{-2} for the consumption of 5 g of *robusta*, mixed, and *arabica* CS, respectively. Instead, the HQ ranged from 2.83×10^{-4} to 1.38×10^{-1}, from 2.29×10^{-4} to 1.09×10^{-1}, and from 1.22×10^{-4} to 1.30×10^{-1} for the consumption of 10 g of *robusta*, mixed, and *arabica* CS, respectively. Since, in all scenarios, the HQs were below 1, non-carcinogenic risk by exposure to a single contaminant could not be attributed to consumption of silverskin to considered levels. In order of magnitude the values in *robusta* CS were: As > Cu > V > Fe > Hg > B > Mn > Ba > Pb > Ni > Zn > Be > Co > Cr. Similarly, the order in mixed CS was V > As > Cu > Fe > Hg > Ba > B > Ni > Mn > Zn > Be > Co > Cr, whereas *arabica* CS reported As > Cd > Mn > Hg > Ba > Cu > Fe > B > Ni> chrysene > Zn > Be > Co > phenanthrene > fluoranthene > Cr.

The HI based on consumption of 5 g and 10 g of CS reported values from 2.96×10^{-1} to 5.93×10^{-1}, from 2.76×10^{-1} to 5.53×10^{-1}, and from 2.34×10^{-1} to 4.67×10^{-1} for *robusta*, mixed, and *arabica* CS, respectively (Figure 1). The two CS varieties and the mixed CS sample showed values of single and cumulative risk simulation <1, indicating a low probability of non-carcinogenic adverse effects.

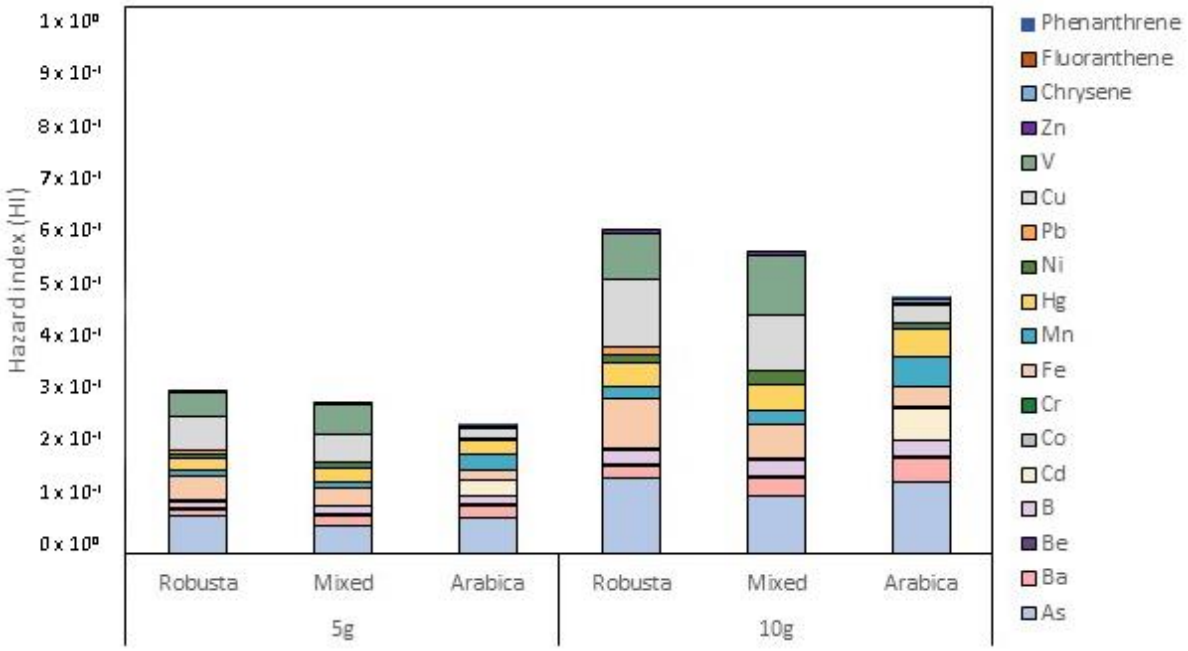

Figure 1. Hazard index (HI) evaluation based on the consumption of 5 g and 10 g of robusta, mixed and arabica silverskin.

Concerning the carcinogenic risk, the estimation of LTCR was only based on As and chrysene since most carcinogenic contaminants were <LOQ. However, the contribution of chrysene was negligible. Instead, As showed a LTCR for the consumption of 5 g and 10 g for *robusta* CS ranged between 2.98×10^{-5} and 5.97×10^{-5}, whereas lower values were reported for mixed CS (2.21×10^{-5} and 4.41×10^{-5}) and *arabica* CS (2.81×10^{-5} and 5.61×10^{-5}). Considering that a carcinogenic risk could occur to values above 1×10^{-4} according to USEPA ISO 6579:2002, the three types of CS showed a low risk to considered dose as an ingredient.

This risk simulation is based on a conservative approach since 100% bioaccessibility and bioavailability were considered, likely overestimating the risk. Indeed, fiber that is highly present in the silverskin may reduce the bioaccessibility of some elements (Hu et al., 2010; Chan et al., 2000; Torre et al., 1991). Furthermore, the slope factors of As refer to inorganic form, although foods may also contain the organic one that is less toxic.

4. Conclusions

An extensive characterization of essential and toxic elements, REE, pesticides, polycyclic aromatic hydrocarbons, and microorganisms on the two main varieties of CS and their blend was provided. In *robusta* and mixed CS pesticides, REE, process contaminants, OTA, and PAHs were not detected. Similar data were reported for the *arabica* CS sample, although chrysene, phenanthrene, and fluoranthene were detected at low concentrations. Essential and toxic elements showed variability among CS varieties and blended, although As was usually the most representative in all samples. Concerning microorganisms, *arabica* and *robusta* CS reported lower contamination than mixed CS, although Salmonella spp. did not occur in any samples. The handling and collection of the coffee silverskin probably denote slight environmental contamination between the mixed CS obtained from coffee companies and that of *arabica* and *robusta* CS obtained in the laboratory. It should be kept in mind that it is a waste material for companies, and, to date, there are no regulations for its proper sorting and collection. However, the high temperatures of the roasting process allow a reduction of CS microbial load. The risk assessment liked the potential consumption of CS as a food ingredient (5 g and 10 g) and pointed out a low probability of non-carcinogenic and carcinogenic effects. *Robusta* CS showed higher values (risk) for HI and LTCR. As noted by comparison with studies in the literature, differences in the chemical profile of CS can be due to coffee variety, production sites, climatic factors, field treatments, coffee processing methods, and, finally, silverskin storage and collection methods. Overall, this study provides the first evidence of the safety of CS based on determinist assessment. However, future studies should assess the bioaccessibility of elements through in vitro or in vivo digestion to provide more data on the healthiness of CS and its potential application as a novel food.

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Coffee Silverskin: Unveiling a Versatile Agri-Food By-Product for Ethical Textile Coatings

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Abstract

In response to the growing focus on sustainability and ethical practices, there is an increasing interest in developing sustainable coated textiles with agri-food by-products as alternatives to traditional animal leather. This transition not only addresses ethical concerns but also contributes to waste reduction within the agri-food industry, fostering a more circular and sustainable approach. The coffee silverskin (CS), a by-product of the coffee processing industry, is gaining significant attention for its potential in sustainable resource optimization and value creation. This study explores the versatility of coffee silverskin as a sustainable coating material for textiles. The innovative exploration of CS as a bio-based coating material involves different residue treatment procedures and formulations to assess its suitability for coatings. The use of planetary ball milling as a pre-treatment method has resulted in a homogeneous deconstruction of the integument, yielding a uniform and soft coating. In conclusion, this study represents a significant step towards sustainable coated textiles as an ethical alternative to animal leather. By harnessing the potential of coffee silverskin, the textile industry can contribute to a more sustainable and responsible future.

Keywords: Coffee Silverskin; Sustainable textiles; Bio-based coatings; Circular economy; Alternative Leather

1. Introduction

In recent years, the growing focus on sustainability and ethical practices has led to an increased interest in (re)utilizing nature residues and developing sustainable coated textiles with food by-products as viable alternatives to the use of animal skin. This shift reflects a collective commitment to reducing environmental impact and promoting responsible sourcing within the textile industry.

The leather industry holds a long-standing history as a traditional manufacturing sector, and its products rank among the most globally traded commodities (Meyer et al., 2021). Leather is considered a bio-based and biodegradable material since its production relies on utilizing by-products of the food industry, specifically derived from meat processing (Ozgunay et al, 2007). However, it is important to note that certain leather varieties are sourced from more exotic animals such as alligators, snakes, and sharks, known for their unique patterns, naturally occurring marks, and distinctive structures (Demeroukas et al., 2015; Wainaina et al., 2022). For this reason, the leather industry has given rise to significant ethical concerns surrounding animal rights and welfare, as well as posing challenges in terms of environmental pollution. The production of leather entails the intricate processing of raw animal skin and hides through a tanning process, necessitating the use of diverse chemicals, such as sodium chloride, ammonium chloride, sulphate, and chromium (Hashmi et al., 2017). Unfortunately, this tanning procedure generates solid, liquid, and gaseous waste, along with the emission of toxic chemicals, leading to significant environmental repercussions (Sivaram et al., 2019). Moreover, these processes expose workers in tanneries to potential health risks associated with carcinogenic compounds (Hashmi et al., 2017).

As the industry continues to evolve, there is an increasing focus on sustainable and ethical alternatives to conventional leather. The utilization of agri-industry residues and food

by-products presents a promising solution to address these challenges. These valuable by-products can be effectively repurposed, either as natural dyes (İşmal Ö. E., 2017). or transformed into bio-based materials suitable for the textile industry (Zhang et al., 2022). By embracing bio-based coatings as alternatives to traditional leather, which are cruelty-free and animal-friendly, the textile industry can proactively address ethical concerns and provide consumers with more ethical choices. Additionally, this transition contributes to the reduction of waste generated in the agri-food industry, fostering a more sustainable and circular approach (Gavahian et al., 2021). Each year, within the European Union (EU), the food and forestry-based industries collectively produce approximately 900 million tonnes of waste (Coelho et al., 2020). Instead of discarding these residues as waste, they can be repurposed to create added value across various applications (Alexandri et al., 2021; Difonzo et al., 2022). In specific, the utilization of bio-based coatings fosters innovation and versatility in the textile industry. These coatings offer flexibility in terms of application methods, customization, and the incorporation of functional properties (Silva et al., 2022). This versatility allows for the development of diverse textile products with improved performance characteristics, unique aesthetics, and enhanced functionality (Zhang et al., 2022).

The agri-food by-products derived from the coffee production chain are currently gathering significant attention and success. This can be attributed to the prominence of coffee as one of the most crucial and commercially valuable chains globally (Barreto Peixoto et al., 2023). The valorization of these by-products exemplify a prime opportunity within the agri-food industry for sustainable resource optimization and value creation. The coffee production generates significant by-products, involves different countries and includes several process steps before obtaining the final well-known beverage. Two major categories of coffee by-products can be distinguished. The pre-roasting by-products, such as skin, pulp, husks,

mucilage and parchment, generated in producing countries and the post-roasting by-products, coffee silverskin and spent coffee grounds, which have a broader distribution (Castillo et al., 2019; Hejna A., 2021). However, through the process of valorization, these by-products can be transformed into valuable commodities, presenting a range of potential applications and benefits (Hoseini et al., 2021; Durán-Aranguren et al., 2021). This study, aligned with the circular economy principles, is focused on the exploration of the versability of the coffee silverskin (CS) as a sustainable material for coating textiles. This by-product is the integument, a thin protective layer, that covers the two hemispheres of the green coffee beans and is detached from them due to the high temperature of the roasting process.

2. Materials and Methods

This study aimed to explore the versatility of coffee silverskin as a sustainable material for developing coated textiles, providing eco-friendly alternatives to natural leather. Different approaches were adopted for each case, and the methodologies used are described below.

2.*1. Sample and Products*

The coffee silverskin (CS) utilized in this study was generously supplied by two coffee industries located in the Campania region of Italy. It was obtained through a blending process involving roasted green beans of Coffea arabica and Coffea robusta (in unspecified ratio). It is common practice for coffee companies to blend different varieties during the roasting process to obtain different aromas and the resulting CS is collected through suction cyclones (Lachenmeier et al., 2022). Different coating pastes were prepared mixing the CS residue with commercial products Impranil ECO DLS and Imprafix 2794 from Covestro (Germany),

Rolflex Bio OP 90 from Lamberti (Italy) and Tubicoat Verdicker LP from CHT (Germany), that were generously supplied.

2.2. Residue pre-treatments

Prior to processing, the coffee silverskin sample was initially ground to a particle size of 0.2 mm using the Retsch SM 300 cutting mill (Retsch GmbH, Haan, Germany). Following this phase, the ground coffee silverskin (CS) underwent two different routes. One path involved its immediate use for the coating formulation (1), as described below. Meanwhile, the other path involved an additional pre-treatment phase utilizing ball milling to further reduce its particle size. To facilitate understanding the various steps, from the treatment of the CS residue to the final evaluations of the obtained coatings, these have been outlined in Figure 1.

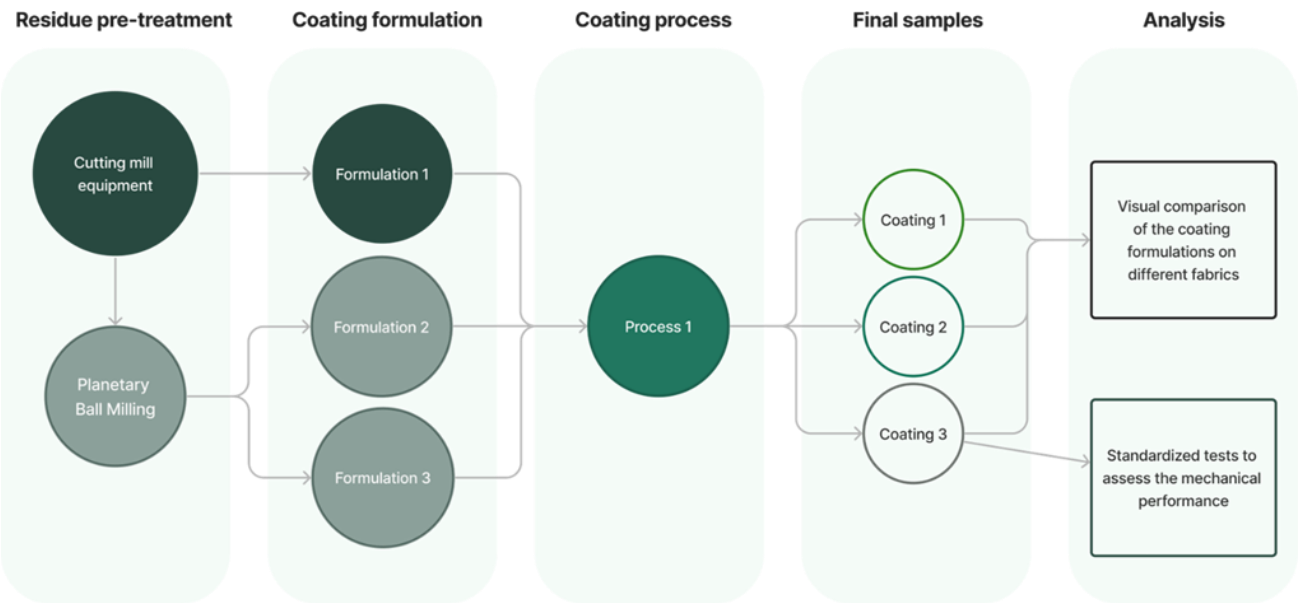

Figure 1. Flow diagram of the steps carried out for the realization of the coatings with coffee silverskin.

Coating formulation (1). The coating paste was formulated by mixing 8 % of the ground CS with Impranil ECO DLS as the polymer base, and Imprafix 2794 as the crosslinker. Tubicoat Verdicker LP was gradually added until a good viscosity of the paste was achieved (~8 Pa.s).

Ball milling treatment. The CS sample, previously ground with the cutting mill equipment, was subjected to a further processing using a Retsch PM100 planetary ball milling (BM) system (Retsch GmbH, Haan, Germany) under wet conditions. The milling process was performed using a 125 mL zirconia milling cup and 5 mm zirconia spheres. The BM treatment lasted for 2 hours at 400 rpm. A mixture of ground coffee silverskin and distilled water was used in a ratio of 1:7. After the BM treatment, a sludge-like consistency was obtained as a result. Utilizing this residue, the coating formulations (2) and (3) were developed.

Coating formulation (2). The coating paste was formulated by mixing an amount of 60 % of the sludge-CS (8 % CS solid content) obtained after the BM treatment with Impranil ECO DLS and Imprafix 2794. Tubicoat Verdicker LP was incrementally incorporated to achieve an optimal texture and workability of the paste.

Coating formulation (3). Another coating paste was formulated by mixing 60 % of the sludge-CS with Impranil ECO DLS and Imprafix 2794.

Coating process. The coating formulations (1), (2), and (3) were applied following the specified process. Cotton and elastane (97:3) fabrics were used as textile substrates for the coatings. The textile substrate was coated using a knife coating process, with the application of three layers of the coating formulations. The first layer had a thickness of <0.01 mm, while the next two layers had a thickness of 0.1 mm. Each layer was dried at 100 °C for 5 minutes.

Following the application of all layers, the substrate was subjected to thermofixation at 150 °C for 5 minutes. In some coating samples, a thin layer (<0.01 mm) of Rolflex Bio OP 90 topcoat was applied. This inclusion aimed to enhance the mechanical properties of the coating while also evaluating its influence on the final aesthetic appearance of the coated substrate.

2.3. Visual comparison of different coffee silverskin coatings

The different coatings obtained were intended for a visual evaluation of their final aesthetic appearance. The primary objective of this experimental phase was to examine how different residue pre-treatment and coating paste formulations could impact the colour and overall aesthetic appeal of the resulting coatings. By conducting this test, valuable insights were obtained concerning the visual characteristics and overall attractiveness of the coated substrates.

2.4. Evaluation of coated substrates

The coatings derived from formulation (3) underwent a comprehensive characterization to evaluate their appropriateness for the intended final application. Prior to the assessment, these coatings were subjected to a calendering process at a temperature of 140 °C and a pressure of 5 bar for 30 seconds. This treatment aimed to further enhance their properties and ensure their suitability for practical usage. To assess the performance of the developed solutions, a series of standardized tests were conducted. These included colour fastness to artificial light: Xenon arc fading lamp test (ISO 105-B02:2014), Martindale Abrasion Resistance (ISO 5470-2:2003), determination of resistance to damage by flexing (De Mattia method and Crumple/Flex) (ISO 7854:1995), colour fastness to water (ISO 105-E01:2013), and colour fastness to rubbing in dry and wet conditions (ISO 105-X12:2016).

3. Results

3.1. Visual comparison of different coffee silverskin coatings

Multiple coatings utilizing coffee silverskin were prepared using various residue treatments and formulations. The primary objective of these coatings was to assess their final visual appearance, as it plays a crucial role in influencing customer perception and preferences.

The outcome of the coatings varied significantly depending on the pre-treatment of the residue and the formulation used for the coating paste, as showed in the Figure 2-3-4. The coating shown in Figure 2, was obtained with the residue without the initial pre-treatment with the Ball milling, following the application of formulation (1). Traces of aggregate granules are visibly present on the surface, which not only impacts the visual appearance of the coating but also affects its texture and feel. The addition of the Rolflex Bio OP 90 topcoat layer has enhanced the texture by providing a slightly matte finish, although it still retains a rough feel. The coating was applied to a cotton and elastane textile substrate (97:3) but considering its particular texture, the coating may be less suitable for garments due to its rough surface.

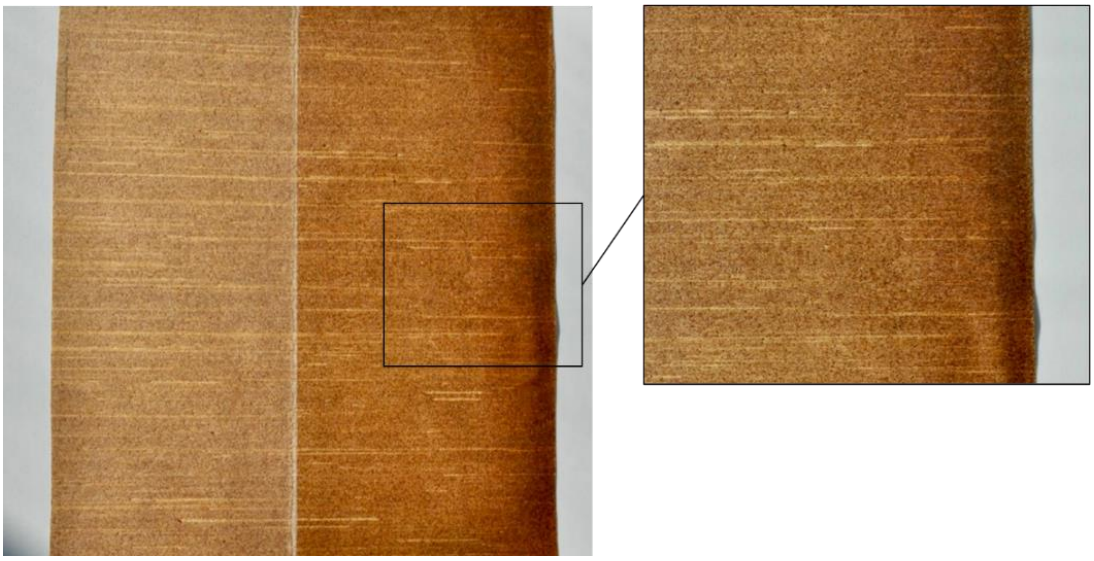

Figure 2. Coating made with coffee silverskin, following the application of formulation 1 on a cotton and elastane (97:3) textile substrate. A zoomed-in view has been provided. In half of the coating sample (left side) a layer of a topcoat was applied.

To address the issue of granules on the coating surface, an additional pre-treatment step was introduced, involving the use of planetary ball milling in wet conditions. The ball milling provides a highly efficient and effective method for size reduction of materials, resulting in the production of smaller particles (Zhang et al., 2014). One of the key advantages of this process lies in the tumbling motion of the balls inside the milling equipment, which promotes efficient mixing of materials. This dynamic mixing action ensures enhanced homogeneity and uniform distribution of components within the final product. Furthermore, the choice of wet treatment conditions utilizing water as a solvent exerts a significant impact on the morphological and structural modifications achieved through ball milling (Avolio et al., 2012). Formulations (2) and (3) of the coating paste were prepared using CS-sludge obtained from the treatment with the planetary ball milling. To maintain an equivalent effective CS solid content as formulation (1), which contained 8 % silverskin, 60 % CS-sludge was

utilized. This CS-sludge proportion allowed to a significant decrease in the total amount of other polymer additives required.

Based on these results, two distinct formulations were developed. The application of the paste obtained from the formulation (2) resulted in an excellent coating with a soft and pleasant texture, showcasing a vivid and uniform dark brown colour with a homogenous layer (Fig. 3). Furthermore, the addition of the topcoat enhanced the coating's texture, giving it a velvety and slightly opaque appearance. The coating exhibited remarkable effectiveness, establishing its potential as a compelling alternative to animal skin, particularly owing to its intense colour.

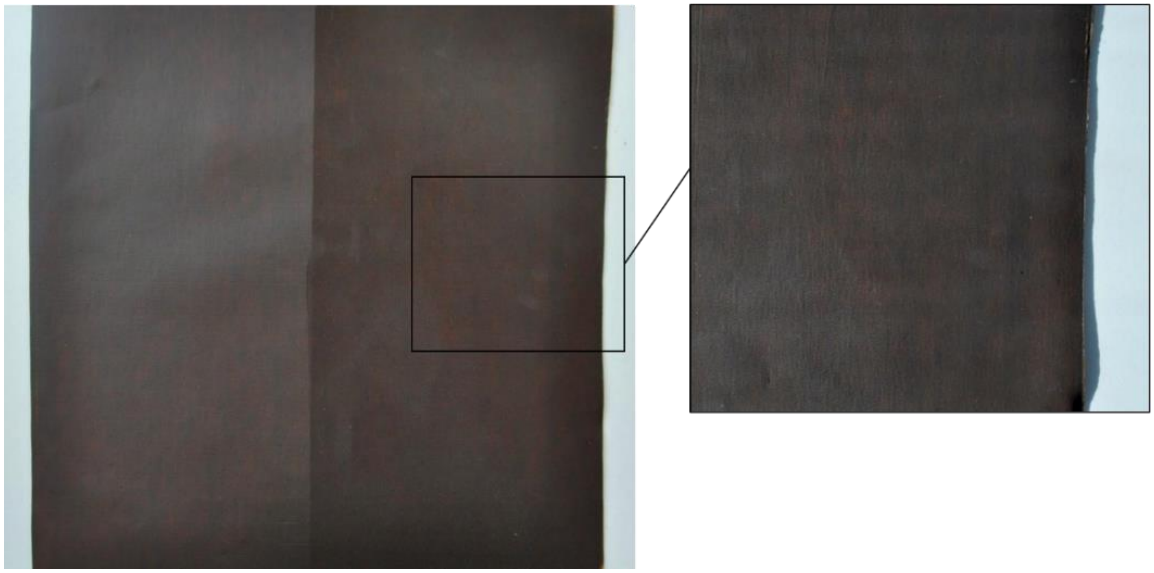

Figure 3. Coating made with coffee silverskin, following the application of formulation 2 on a cotton and elastane (97:3) textile substrate. A zoomed-in view has been provided. In half of the coating sample (left side) a layer of topcoat was applied.

The primary goal of the third formulation (3) was to maximize the economic and environmental sustainability of the coating by reducing the number of polymers used. This formulation exclusively utilized water-based polymers. The resulting coating (Fig. 4)

demonstrated outstanding outcomes in terms of texture and visual appeal. The colour of the coating appeared as a lighter brown, reminiscent of the natural appearance of animal skin, offering a visually appealing alternative. The coating exhibits a consistent and soft texture to the touch, while visually displaying unique variations due to the inherent natural colour of the coffee silverskin, rendering each coating distinct and unique. It is evident from this outcome that the polymers incorporated in formulation (2) enhance the colours, imparting vividness and brilliance to the coatings. Similarly, the application of the topcoat layer on the coating surface resulted in a smoother finish, further enhancing its soft and velvety touch.

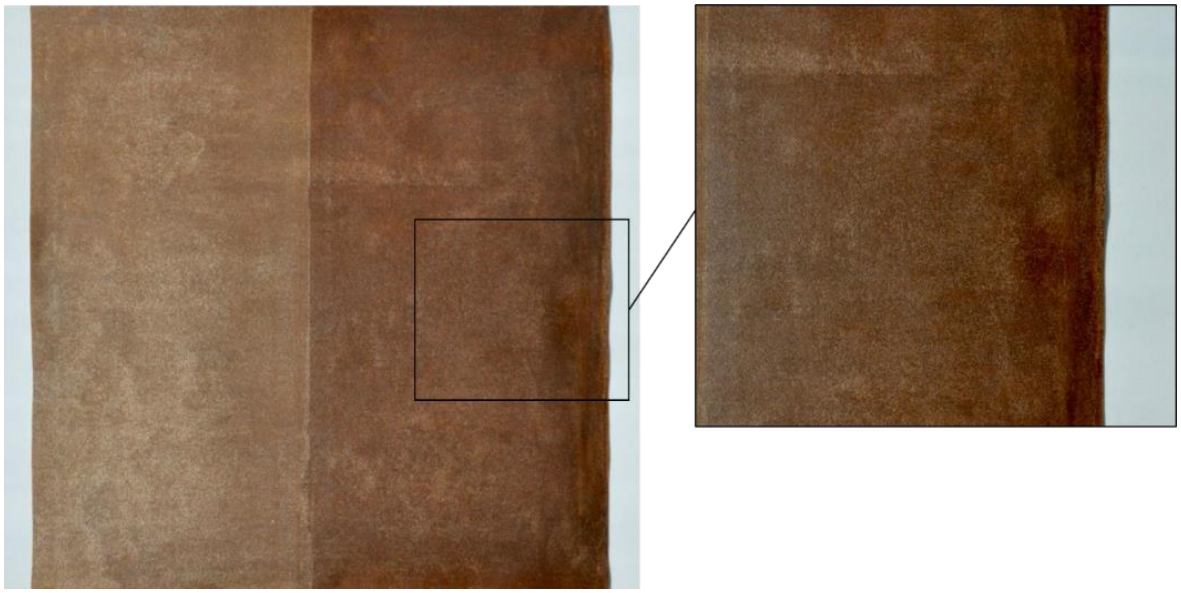

Figure 4. Coating made with coffee silverskin, following the application of formulation 3 on a cotton and elastane (97:3) textile substrate. A zoomed-in view has been provided. In half of the coating sample (left side) a layer of topcoat was applied.

3.2. Evaluation of coated substrates

In light of the remarkable outcomes achieved by this latter sustainable formulation, a comprehensive series of standardized tests (section 2.4 of Materials and Methods) was performed. By conducting these standardized tests, the mechanical properties, durability, and

colour fastness of the coated substrates can be evaluated, providing valuable information to ensure their suitability for practical applications. The corresponding results are presented in Table 1.

Table 1. Results of standardized tests on coffee silverskin coating (formulation 3)

Standardized Tests		Values
Colour Fastness to Artificial Light (ISO 105-B02:2014)		1-2 – Low fastness
Martindale Abrasion Resistance (ISO 5470-2:2003)		3 – Moderate alteration
Determination of Resistance to Damage by Flexing (ISO 7854:1995)	De Mattia method	0 - No alteration
	Crumple/Flex method	1 – Slight alteration
Colour Fastness to Rubbing (ISO 105-X12:2016)	Dry conditions	4-5 – High fastness
	Wet conditions	2-3 - Moderate alteration
Colour Fastness to Water (ISO 105-E01:2013)		4 – Good fastness

The Colour Fastness to Artificial Light (ISO 105-B02:2014) evaluates the resistance of the coating to fading or discoloration when exposed to simulated sunlight using a Xenon arc fading lamp. It helps determine the coatings' colour stability and durability under prolonged exposure to light. The European scale consists of 8 strips of blue dyed wool fabric, numbered from 1 (very low fastness) to 8 (very high fastness). The values obtained from this analysis show a low fastness to artificial light. The Martindale Abrasion Resistance (ISO 5470-2:2003) assesses the resistance of the coating to abrasion and wear caused by rubbing or friction. It simulates everyday usage scenarios, allowing to gauge the coatings' ability to withstand wear and maintain their appearance over time. The result obtained on the coating showed moderate alterations. The determination of Resistance to Damage by Flexing (De Mattia method and Crumple/Flex) (ISO 7854:1995) evaluate the flexibility and resistance of the coating to

damage when subjected to repeated bending or flexing. It helps assess its ability to endure mechanical stress without cracking or losing integrity. In both methods, the tested coating exhibited minimal alterations, even after undergoing a substantial number of cycles, amounting to 150,000 and 9,000 for the De Mattia and Crumble/Flex method, respectively. These results indicate significant flexural strength. Colour Fastness to Rubbing in Dry and Wet Conditions (ISO 105-X12:2016) test evaluate the colour fastness of the coating when rubbed against different materials. The test was assessed in dry and wet conditions. It helps assess how the coatings hold up against friction, which is essential for maintaining their appearance and performance during everyday use. The strength ratings, based on the Grey Scale for staining, vary from 1 (poor rating) to 5 (better rating). Results from this test demonstrate that the coating exhibits excellent colour fastness under dry conditions, with ratings of 4-5. However, when the sample is exposed to wet conditions, more noticeable changes in colour are observed. The Colour Fastness to Water (ISO 105-E01:2013) measures the ability of the coating to resist colour change or bleeding when exposed to water. A multifibre fabric is used to assess colour transfer and the change in colour of the coating is assessed using the Grey Scale. From the test conducted, the coating exhibits a good fastness to water.

These results are essential for manufacturers and designers to ensure that the textiles meet the specific performance requirements. Additionally, the test results can be used to compare different textile materials and make informed decisions during the product development process. It is important to highlight that the subsequent analyses were conducted on the coating without the topcoat layer. Incorporating an additional protective layer could significantly enhance the coating's performance, particularly in terms of resistance to abrasion

and colour fastness to rubbing. This improvement would provide added durability and stability to the coating, making it more suitable for demanding applications.

4. Discussion

Coffee silverskin has gathered significant attention due to its promising potential for reuse in various sectors. Its nutritional and chemical composition has piqued the interest of the food and nutraceutical industries (Nzekoue et al., 2022; Machado et al., 2023). Furthermore, the morphological and structural characteristics of the integument have demonstrated promising potential in developing innovative packaging solutions (Garcia et al., 2021). From a technological perspective, coffee silverskin has emerged as one of the most promising by-products within the coffee industry (Hejna A., 2021). This is primarily attributed to its exceptionally low moisture content (~7-10%) and a relatively high protein content (~18-20%), which can serve as plasticizers or offer opportunities for enhanced interfacial adhesion with polar polymer matrices due to the presence of functional groups (Hejna A., 2021; Toschi et al., 2014). In a preliminary study on the characterization of coffee silverskin (Nolasco et al., 2022), Scanning Electron Microscopy (SEM) analysis revealed a compact and wrinkled surface, indicating that the fibrous structure of cellulose is surrounded by a matrix of less structured components like hemicellulose/lignin. At higher magnification, near-spherical particles ranging from approximately 0.5 to 5 μm were observed on the integument's surface, potentially attributed to the lignin and/or protein fraction. By utilizing planetary ball milling, the coffee silverskin's structural integrity was effectively altered, promoting a more uniform and consistent coating. Furthermore, the mechanical treatment may have introduced modifications to the integument's composition, enhancing its ability to form strong bonds with polar polymer matrices due to the presence of functional groups within its

protein content (Hejna A., 2021). This unique combination of uniformity and enhanced adhesion potential opens exciting possibilities for its application in sustainable and innovative coated textile solutions. Furthermore, the conducted standardized tests revealed interesting results concerning the coating's resistance to bending, breakage, and colour stability under diverse stress conditions. These results indicate the potential of the coating to withstand various mechanical and environmental challenges, making it a promising candidate for practical applications.

5. Conclusions

Developing sustainable coated textiles with food by-products aligns with the principles of environmental conservation and resource efficiency. By minimizing the use of animal skin and opting for bio-based coatings, the industry can reduce its reliance on resource-intensive processes associated with traditional leather production. While a complete replacement of leather with bio-based coatings may not be feasible for all applications or preferences, their utilization represents a significant stride towards a more sustainable, ethical, and environmentally conscious textile industry. This study focuses its investigation on the coffee roasting by-product, aiming to assess its viability as a coating material, its mechanical properties, and its resistance against various stress conditions through standardized tests. The novelty of this research lies in exploring an untested waste material within the textile industry. Additionally, the utilization of ball milling as a pre-treatment step emerges as a valuable tool to achieve a more uniform coating, allowing the formulation of a coating paste with a smaller amount of polymers. The results conducted on the coffee silverskin coating indicate suitable mechanical durability but limited colour fastness when subjected to light exposure. Further studies will be undertaken to enhance the coating's potential, while preserving its visual

appearance and tactile qualities. This study paves the way for interesting research into the reuse of unexplored waste materials from the food chain and the promotion of innovative and sustainable approaches to textile production.

Acknowledgement

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Conclusions and future perspectives

This doctoral project focuses on elevating the role of Coffee Silverskin (CS), traditionally discarded during coffee roasting, into valuable resources. The thesis, divided into seven chapters, systematically explores the sustainable applications of CS within the coffee supply chain, offering promising paths for functional foods, bioconversion of insects, bio-coatings for packaging and sustainable alternatives in the textile industry.

Reviewing the thesis structure, the following potential future perspectives are suggested.

- ✓ The findings in Chapter I open avenues for further research in utilizing CS for nutrition, nutraceuticals, and industrial applications. Investigating specific bioactive peptides and optimizing their extraction processes could enhance the nutritional and industrial value of CS.

- ✓ Chapter II's assessment of CS in the food sector indicates the potential for developing functional foods. Future research can focus on refining formulations and exploring innovative ways to incorporate CS into a broader range of functional food products.

- ✓ …

- ✓ …

- ✓ The exploration of CS in the textile industry, as discussed in Chapter V, VI and VII unveiled the potential of CS as a valuable biomaterial for textile coatings. Ongoing

research can further optimize treatment procedures and formulations, exploring new possibilities for sustainable alternatives in the textile industry.

In conclusion, this sustainable journey not only emphasizes the importance of reusing agricultural waste by opening new possibilities for collaboration between different sectors but also reveals the different and emerging roles that Coffee Silverskin can play in shaping a more conscious future for both the environment and resources.