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Coffee silverskin: characterization of B-vitamins, macronutrients, minerals and phytosterols

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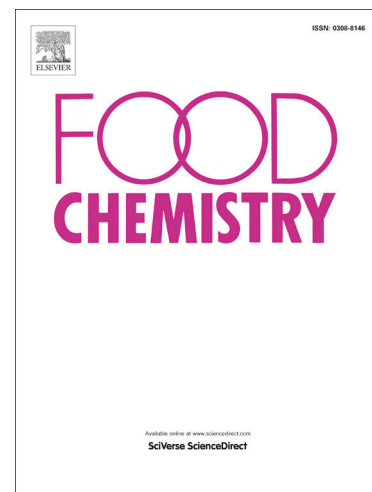
1 **Coffee silverskin: characterization of B-vitamins, macronutrients, minerals and**

2 **phytosterols**

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5 Franks Kamgang Nzekoue[a], Germana Borsetta[a], Luciano Navarini[b], Doaa Abouelenein[a,c], Jianbo

6 Xiao[d,e], Gianni Sagratini[a], Sauro Vittori[a,f], Giovanni Caprioli[a*], Simone Angeloni[a,f]

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9 [a] School of Pharmacy, University of Camerino, via Sant' Agostino 1, 62032 Camerino, Italy

10 [b] illycaffè S.p.A., via Flavia 110, Trieste I-34147, Italy

11 [c] Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt

12 [d] Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science,

13 Faculty of Food Science and Technology, University of Vigo-Ourense Campus, E-32004 Ourense,

14 Spain

15 [e] School of Food and Biological Engineering, Chengdu University, Chengdu 610106, Sichuan,

16 China.

17 [f] RICH – Research and Innovation Coffee Hub, via E. Betti 1, I-62020, Belforte del Chienti (MC),

18 Italy

19

20

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22 ***Corresponding author**: Giovanni Caprioli, School of Pharmacy, University of Camerino, via

23 Sant' Agostino 1, 62032 Camerino, Italy. Phone: +39 0737402238. Fax: +39 0737637345. Email:

24 giovanni.caprioli@unicam.it

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Abstract

The present study assessed the nutritional composition of coffee silverskin (CSS) obtained from arabica roasted coffee. Following validated analytical methods, CSS resulted to be a high source of proteins (14.2 g/100 g) and dietary fibers (51.5 g/100 g). Moreover, the mineral analysis revealed high contents of calcium (1.1 g/100 g) and potassium (1.0 g/100 g). To date, this study provided the widest mineral profile of CSS with 30 minerals targeted including 23 microminerals with high levels of iron (238.0 mg/kg), manganese (46.7 mg/kg), copper (37.9 mg/kg), and zinc (31.9 mg/kg). Moreover, vitamins B_2 (0.18−0.2 mg/kg) and B_3 (2.5−3.1 mg/kg) were studied and reported for the first time in CSS. β-sitosterol (77.1 mg/kg), campesterol, stigmasterol, and Δ5-avenasterol, were also observed from the phytosterol analysis of CSS with a total level of 98.4 mg/kg. This rich nutritional profile highlights the potential values of CSS for innovative reuses in bioactive ingredients development.

1. Introduction

Coffee silverskin (CSS) is the integument of coffee beans, which is released as the main by-product of the coffee roasting process. Considering the high production and consumption of coffee, thousands of tonnes of CSS are produced each year in coffee-roasting industries worldwide (Juan-García et al., 2021). Indeed, according to the International Coffee Organization (ICO) statistics, around 9.9 billion kg of coffee are yearly consumed (ICO, 2021), corresponding to 200−400 million kg of CSS generated (Barbero-López et al., 2020; del Pozo et al., 2020). Moreover, according to FAO, growth projections on coffee production and consumption are expected to increase over the next years. In 2019 the world production accounted for 10,035,576 tonnes with a harvested area of 11,120,498 hectares, and data compared to 2009 respectively account for + 1.5% in production and + 6.31% in the harvesting surface (FAO, 2019).

At the global level, the significant amount of discards in the coffee industry (23 million tons of waste per year) is leading to a greater and greater valorization of such food wastes, becoming a priority research line to achieve a more sustainable food value-chain, in line with the growing attention of consumers and the international community to sustainability and resilience in the agri-food sector (Conrad, Tichenor, and Blackstone, 2021).

Wastes generated from the coffee industries have become a resource, especially in agricultural contexts. For example, spent coffee grounds are used with a «Zero Wastes» approach with different applications in agriculture, such as fertilizer for growing mushrooms, as they contain minerals and nutrients that are useful for their growth. Other applications contemplate the use of coffee wastes to create pellet or to produce biobased materials such as pens and cups.

In addition, Therefore various studies have been carried out to develop innovative solutions and propose value-added applications for the valorization of CSS according to principles of the green and circular economy. The most recent studies on CSS valorization described the conversion of CSS into different value-added products including biopolymers composite for packaging development (Garcia & Kim, 2021), feedstock for wood preservative formulation (Barbero-López

78 et al., 2020), ingredients for functional foods formulation (Gemechu, 2020), and functional

79 ingredients in cosmetic products (dos Santos et al., 2021).

80 These applications can be performed thanks to the chemical characteristics and composition of CSS.

81 Indeed, CSS is reported to be a high source of dietary fibers and phytochemicals including

82 chlorogenic acids, melanoidins, and caffeine (Castaldo et al., 2020). Various studies assessed the

83 bioactive compounds and the nutritional composition of CSS giving more attention to the fibers

84 composition, fat profile, proteins, and polyphenols content (del Pozo et al., 2020). Other classes of

85 bioactive compounds in CSS have not been comprehensively assessed including minerals, vitamins,

86 and phytosterols.

87 The mineral profile of CSS has been discussed in few studies focusing principally on the most

88 important macrominerals (Wen et al., 2020). Nevertheless, microminerals and toxic heavy metals,

89 which are health-impacting elements have not been considered. Similarly, phytosterols, which are

90 cholesterol-lowering bioactive compounds have been poorly assessed in CSS. Indeed, to the

91 author's knowledge, the characterization and the content of phytosterols in CSS have been

92 performed in just one study (Toschi et al., 2014). However, the sample studied was obtained from a

93 mixture of arabica and robusta coffee. Considering vitamins, the related literature is scarce. The

94 vitamin E profile of CSS has been investigated by Costa et al., 2018 (4.3 mg/100 g) and Bessada et

95 al., 2018 (5.3–16.8 mg/100 g). Besides, B-vitamins, which some of them derive from the thermal

96 degradation of trigonelline could be abundant micronutrients in CSS. Indeed, trigonelline is an

97 important alkaloid in coffee (averagely 1 g/100 g DW) that can be highly degraded during roasting

98 (50–80% of decomposition) (Gemechu, 2020). However, to date, no study has assessed the profile

99 of B-vitamins in CSS.

100 Therefore, the present study aims to perform a comprehensive analysis of nutrients, microelements,

101 and bioactive compounds in CSS. The analyses will be focused on non-assessed and poorly studied

102 compounds (minerals, B-vitamins i.e., riboflavin and niacin, and phytosterols) in order to propose

103 novel value-added applications to CSS.

2. Materials and methods

2.1 Chemicals and reagents

Riboflavin (purity > 98%, $C_{17}H_{20}N_4O_6$, molecular weight 376.4 g/mol, CAS Number 50-81-7), nicotinamide (purity > 99.5%, $C_6H_6N_2O$, molecular weight 122.1 g/mol, CAS Number 98-92-0), nicotinic acid (purity > 99.5%, $C_6H_5NO_2$, molecular weight 123.1 g/mol, CAS Number 59-67-6), and β-sitosterol ($C_{29}H_{50}O$, molecular weight 414.7 g/mol, CAS Number 83-46-5) were purchased from Sigma-Aldrich (Milan, Italy). Campesterol ($C_{28}H_{48}O$, molecular weight 400.7 g/mol, CAS Number 474-62-4), Δ5-avenasterol ($C_{29}H_{48}O$, molecular weight 412.7 g/mol, CAS Number 17605-67-3), and stigmasterol ($C_{29}H_{48}O$, molecular weight 412.7 g/mol, CAS Number 83-48-7) were provided by Phytolab (Vestenbergsgreuth, Germany). Individual stock solutions of nicotinamide and nicotinic acid were prepared by dissolving 10 mg of pure powder into 10 mL of acetic acid 2% (*v/v*) solution, while stock solutions of phytosterol standards were prepared in chloroform (10 mg/ml). On the other hand, the stock solution of riboflavin was obtained by dissolving 4 mg of the pure standard into 100 mL of acetic acid solution. All stock solutions were stored in a glass-stoppered bottle at 4 °C in the dark and different solution concentrations of the three analytes (standard working solutions) were daily prepared by diluting appropriately the stock solutions in the acetic acid solution for B-vitamins, and methanol for phytosterols. Ultra-pure water was obtained using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA) while HPLC-grade acetonitrile was purchased from Sigma-Aldrich (Milan, Italy). HPLC-grade formic acid 99–100% was bought from J.T. Baker B.V. (Deventer, Holland). All other chemicals were analytical grade. Before liquid chromatography-mass spectrometry (LC-MS) analysis all samples were filtered with Phenex™ RC 4 mm 0.2 μm syringeless filter, Phenomenex (Castel Maggiore, Italy).

2.2 Coffee silverskin collection and preparation

129 Coffee silverskin (CSS), which was collected after roasting green beans (*Coffea arabica* L.) at

130 medium/dark level, was kindly provided by Illycaffe S.p.A. (Trieste, Italy). Before extraction and

131 analysis, CSS was frozen with liquid nitrogen and then milled with an Ariete Blendy 570 grinder

132 (Florence, Italy) to obtain a homogeneous powder that was stored at 4 °C, at dark, until use.

133 **2.3 Macronutrient and fiber analysis~~Nutritional analysis and mineral content~~**

134 CSS samples were analyzed to assess their chemical composition through the AOAC procedures.

135 The moisture content was determined by drying the sample in the oven (24 h, 133 °C) until steady

136 weight. The crude fat level was obtained by the Soxhlet extraction of a fixed quantity of CSS

137 sample with petroleum ether; the protein content was estimated through the Kjeldahl method; the

138 ash content was determined by incineration at 600 ± 15 °C; dietary fiber levels were evaluated

139 through a gravimetric method after acidic hydrolysis, while total carbohydrates were calculated by

140 the difference.

141 **2.4 Mineral analysis**

142 The mineral composition was performed by the digestion of dried samples with nitric acid

143 (Suprapur, Merck) followed by analysis with inductively coupled plasma mass spectrometry (ICP-

144 MS, Agilent Technologies, Santa Clara, CA) (Nkuimi et al. 2019).

145 **2.5 Vitamin B_2 and B_3 analysis ~~2.4 Vitamin B_2 and B_3 extraction and LC–MS quantification~~**

146 The extraction of vitamin B_2 (riboflavin) and B_3 (niacin, i.e., nicotinic acid and nicotinamide) was

147 performed taking into consideration a paper by Caprioli, Sagratini, Vittori, and Torregiani (2018),

148 who evaluated the efficiency of different extraction procedures for these vitamins. For the current

149 research was selected only the most efficient procedure obtained in that research, in terms of

150 recoveries, quantitative results obtained, and rapidity but varying the solvent-to-sample ratio (SSR)

151 such as 20:1, 30:1, and 40:1 (v/w, mL/g). The extraction of nicotinamide, nicotinic acid, and

152 riboflavin was carried out by adding 1 g of CSS powder together with 0.1 M hydrochloric acid

153 (HCl) into a 100 mL flask and the hydrolysis was performed at 100 °C for 30 min. Samples were

154 then cooled down at room temperature and were filtered with Whatman filter paper; the solution pH

155   was later adjusted to 4.0−4.5 by adding an appropriate amount of 2 M sodium acetate. Finally, it

156   was diluted to 50 mL with ultra-pure water, and before high-performance liquid chromatography-

157   tandem mass spectrometry (HPLC-MS/MS) analysis, the sample was centrifuged at 13,000 rpm for

158   10 min and filtered with a 0.2 μm membrane filter.

159   The quantification of niacin and riboflavin was performed following a previous procedure (Caprioli

160   et al., 2018). Briefly, an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent

161   Technology (Santa Clara, CA) were employed for the current research. The MS system was

162   composed of an electrospray ionization (ESI) source which operated in positive ionization mode.

163   The separation of the target compounds was achieved using a Kinetex HILIC analytical column

164   (100 mm× 4.6 mm i.d., particle size 2.6 μm) from Phenomenex (Torrance, CA, USA). The mobile

165   phase was composed of water (A) and acetonitrile (B) both with 0.1% of formic acid and the flow

166   rate was 0.8 mL/min. The elution was achieved in isocratic condition and the injection volume was

167   2 μL. The source parameters were as following: temperature of the drying gas, 300 °C, gas flow, 12

168   L/min, nebulizer pressure, 50 psi, and capillary voltage, 4,000 V. The acquisition was performed in

169   "Multiple Reaction Monitoring" (MRM) mode and the most abundant transitions were used for

170   quantitation while the other for confirming the analyte presence. The HPLC-MS/MS acquisition

171   parameters including the selected mass transitions and retention time (Rt) are shown in **Table 1**.

172   **2.5.1 Method validation**

173   The analysis of vitamin B_2 (riboflavin) and B_3 (nicotinic acid and nicotinamide) has been carried

174   out following a developed method (Caprioli et al., 2018) which used a hydrophilic interaction

175   chromatography (HILIC) instead of classic reversed-phase (RP) since HILIC is more suitable for

176   polar compounds as hydrosoluble vitamins are, and it enhances compatible with MS system (Porter,

177   & Lodge, 2021). Before the analysis of CSS, the analytical procedure has been validated by

178   assessing the linearity, sensitivity, repeatability, and recovery (**Table 2**). The linearity was

179   evaluated by injecting eight different concentrations of standard mixtures and preparing calibration

curves. These were constructed by plotting the standard peak areas by concentrations and for each analyte, a determination coefficient (R^2) was obtained. All analytes showed good linearity since the R^2 was ≥ 0.9945. The sensitivity has been studied by measuring the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were estimated by injecting low concentrations of studied compounds and the concentration that generated a signal-to-noise ratio (SNR) of 3 was assigned to LOD while that with SNR of 10 was designated as LOQ. The analytes showed satisfactory sensitivity since LOQ was 3 ng/mL for all vitamins and these values were similar to those reported in the literature (Caprioli et al., 2018). The method repeatability was evaluated by injecting five times three analyte concentrations in the same day and during three consecutive days and the intraday and interday repeatability was calculated and expressed as relative standard deviation (%RSD). The intraday and interday repeatability were 2.5–4.6% and 3.9–6.2%, respectively. Recovery studies were performed at two different spiking levels, 1 and 0.1 µg/g to study the accuracy of the current method. These studies have been evaluated for each SSR level but similar findings have been obtained. **Table 2** shows the recoveries of the procedure which employed SSR of 30:1 and satisfactory recovery levels were found with both spiking levels for all analytes such as 78–86% with 0.1 µg/g and 82–93% with 1 µg/g.

2.6~~5~~ Phytosterols analysis~~Analysis of phytosterols~~

The analysis of phytosterols (PS) required a prior extraction step, which was performed following the method of Nzekoue et al. (2020). Briefly, 1 g of CSS was submitted to acidic hydrolysis with 4 mL of HCl solution (0.25 N) through 10 min of ultrasonication. The extraction was followed by a saponification process at 80 °C for 40 min adding 5 mL of KOH (50 g/100 mL) and 20 mL of ethanol. Then, PS were extracted with 10 mL of hexane three times (10 mL x 3). The collected extract was dried and then derivatized (40 °C, 30 min) with 1 mL of dichloromethane containing 4-dimethylaminopyridine (DMAP) and dansyl chloride (8 mg/mL). Derivatized samples were dried, reconstituted with 1 mL acetonitrile, and filtrated with a 0.45 µm membrane filter before analysis.

The identification and quantification of PS were performed through HPLC analyses, using a 1260 Infinity LC-DAD system (Agilent Technologies, Santa Clara, CA, USA). Analyses were carried out in isocratic mode with methanol (100%) as mobile phase at a flow rate of 0.5 mL/min. Analytes separation was performed through a Gemini C18 column (250 × 3.0 mm, 5 μm) preceded by a security guard column C18 (4 × 3 mm, 5 μm), (Phenomenex, Torrance, CA, USA). The volume of injection was 20 μL, and the wavelength of detection was 254 nm.

The analytical method was validated (**Table S1**) showing a good linearity ($R^2 \geq 0.995$) for all the 4 detected phytosterols at a concentration range from 0.5 to 100 μg/mL. Moreover, the reproducibility of the method was determined calculating the relative standard deviation (%RSD) between 3 consecutive analysis of a mix standard sample (50 μg/mL) on the same day (intraday) and during 3 consecutive days (interday). The intraday reproducibility was between 0.1% and 1.8% while the interday reproducibility ranged between 1.2 – 6.3% confirming thus the reproducibility of the method. The assessment of the LOD (9-25 ng/mL) and the LOQ (29-83 ng/mL) showed the high sensibility of the analytical method.

2.76 Statistical analyses

All the experiments were carried out in three replicates (*n*=3) and the results are expressed as mean ± standard deviation (SD). Relative standard deviation (%RSD) was determined between replicates to assess the precision of the obtained results by using Microsoft Excel (Microsoft Office 2019).

3. Results and discussions

3.1 Macronutrients and fiber composition of CSSNutritional composition

The nutritional composition (moisture, carbohydrates, fat, protein, dietary fiber, and ash content) of CSS is reported in **Table 32**. CSS is mainly composed of dietary fibers (51.5 ± 9.7 g/100 g), which are important components for functional foods dedicated to promoting the digestive system health and reduce the risk of chronic diseases such as cardiovascular diseases and type-2 diabetes (Yegin, Kopec, Kitts, and Zawistowski, 2020). Moreover, CSS resulted to be high in proteins with a total

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protein content of 14.2 ± 1.0 g/100g. CSS could thus represent a novel source of dietary proteins to address the global protein challenge associated with the growing population (Weindl et al., 2020).

In addition, CSS shows relatively low levels of fats (3.7 ± 0.3 g/100 g) and consistent ash content (4.9 %). Furthermore, CSS shows also a low moisture content (7.70 ± 0.46 %), which is reported to facilitate the grinding process and allow the production of small particle sizes with reduced energy (Moon & Yoon, 2018). These characteristics are particularly advantageous for possible industrial transformations of CSS.

The nutritional composition of CSS is consistent with the range of levels reported in the literature. Indeed, Wen et al (2020) reported 14.6 g/100 g of proteins and fat content of 3.6 g/100 g in CSS, while Ballesteros, Teixeira, and Mussatto (2014) obtained dietary fiber levels of 54.1 g/100 g.

3.2 Mineral composition of CSS

The analysis of minerals was also performed for a total of 30 targeted minerals including 7 macrominerals (calcium, chloride, magnesium, phosphorus, potassium, sodium, and sulfur). To our knowledge, the present study reports the most complete mineral composition of CSS with a total of 30 quantified minerals (**Table 32**).

Analyses highlighted that CSS possesses high levels of calcium (1,080 mg/100 g) and can be exploited for the production of calcium ingredients for food or pharmaceutical applications. Indeed, calcium is an essential element for various physiological functions including neurotransmission, blood clotting, cell division, muscle activity, and bone and teeth maintenance (Cormick et al., 2021). Moreover, CSS showed high levels of potassium (972 mg/100 g). This macromineral plays a key role in the maintenance of blood pressure, the nervous system, and muscle functions (Udensi & Tchounwou, 2017). In addition, CSS resulted to be a good source of magnesium being the third most abundant mineral of CSS (257 mg/100 g). Magnesium is an essential mineral reported to play numerous roles in the normal functioning of the human body such as energy production, muscle contraction, protein synthesis, bone growth, and blood pressure (Al Alawi, Majoni, & Falhammar, 2018). Appreciable levels of chloride (12.3 mg/kg), phosphorus (12.4 mg/kg), sodium (110 mg/kg),

257 and sulfur (51.9 mg/kg) were also observed. Although some differences related to the studied coffee

258 varieties, calcium, potassium, and magnesium are reported in similar studies to be the most

259 abundant minerals in CSS. Ballesteros et al. (2014) reported higher levels of potassium (2.11 g/100

260 g) in CSS, which was more abundant than calcium (0.94 g/100 g) and magnesium (0.31 g/100 g).

261 Contrarily, Wen et al. (2020) obtained a more similar order with calcium, potassium, and

262 magnesium levels of 1.48, 1.21, and 0.05 g/100 g respectively.

263 Concerning microminerals, 23 elements were assessed with a total concentration of 540.4 mg/kg in

264 CSS. Iron resulted to be the most concentrated micromineral in CSS with levels of 238 mg/kg

265 followed by aluminum (89.0 mg/kg). Iron is an important micronutrient playing key roles in red

266 blood cell formation, cell division, fatigue reduction, immune system, and cognitive functions

267 (Finkelstein, Haas, & Mehta, 2017). In addition, high levels of essential micronutrients were also

268 observed including manganese (46.7 mg/kg), copper (37.9 mg/kg), zinc (31.9 mg/kg), boron (26.1

269 mg/kg), and molybdenum (0.2 mg/kg). These results are consistent with the study of Ballesteros et

270 al. (2014). Copper and manganese contribute to bone health maintenance, oxidative stress

271 modulation, and connective tissue formation. In addition, copper supports the nervous and immune

272 systems and is essential for the absorption and transport of iron (Cámara et al., 2021). Boron is

273 reported to be involved in various functions such as bone growth and maintenance, magnesium

274 absorption, and oxidative stress reduction (Pizzorno, 2015). Zinc is an essential micronutrient

275 supporting a wide number of functions including metabolism, bone health, immune system, cell

276 division, protein synthesis, cognitive function, reproduction, and cell protection from oxidative

277 stress (Cakmak & Kutman, 2018).

278 However, significant levels of potentially toxic and carcinogenic heavy metals were also observed

279 including arsenic (0.13 mg/kg), cadmium (0.07 mg/kg), lead (0.25 mg/kg), or mercury (0.05

280 mg/kg), which can cause various health hazards. Therefore, it is necessary to apply decontamination

281 protocols using chelating agents for the removal of heavy metals before the valorization of CSS in

282 food or nutraceutical products.

3.3 ~~Content of Vitamin B$_2$ and B$_3$ in CSS~~ B-vitamins analysis in CSS

~~3.3.1 Analytical method validation~~

~~The analysis of vitamin B$_2$ (riboflavin) and B$_3$ (nicotinic acid and nicotinamide) has been carried out following a developed method (Caprioli et al., 2018) which used a hydrophilic interaction chromatography (HILIC) instead of classic reversed-phase (RP) since HILIC is more suitable for polar compounds as hydrosoluble vitamins are, and it enhances compatible with MS system (Porter, & Lodge, 2021). Before the analysis of CSS, the analytical procedure has been validated by assessing the linearity, sensitivity, repeatability, and recovery (**Table 3**). The linearity was evaluated by injecting eight different concentrations of standard mixtures and preparing calibration curves. These were constructed by plotting the standard peak areas by concentrations and for each analyte, a determination coefficient (R^2) was obtained. All analytes showed good linearity since the R^2 was $\geq$ 0.9945. The sensitivity has been studied by measuring the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were estimated by injecting low concentrations of studied compounds and the concentration that generated a signal-to-noise ratio (SNR) of 3 was assigned to LOD while that with SNR of 10 was designated as LOQ. The analytes showed satisfactory sensitivity since LOQ was 3 ng/mL for all vitamins and these values were similar to those reported in the literature (Caprioli et al., 2018). The method repeatability was evaluated by injecting five times three analyte concentrations in the same day and during three consecutive days and the intraday and interday repeatability was calculated and expressed as relative standard deviation (%RSD). The intraday and interday repeatability were 2.5–4.6% and 3.9–6.2%, respectively. Recovery studies were performed at two different spiking levels, 1 and 0.1 µg/g to study the accuracy of the current method. Satisfactory recovery levels were found with both levels for all analytes such as 78–86% with 0.1 µg/g and 82–93% with 1 µg/g.~~

~~3.3.2 Content of B-vitamins~~

This is the first study that investigated the content of vitamin B$_2$ (riboflavin) and B$_3$ (niacin) in CSS.

Table 4 reports the content, expressed as µg/g, of riboflavin (vitamin B$_2$), nicotinic acid,

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309 nicotinamide, and niacin (vitamin B$_3$) found in coffee silverskin. As an example, **Figure 1** reports

310 the HPLC-MS/MS chromatograms of a standard mixture at 100 ng/mL and a CSS sample. Three

311 different SSR such as 20:1, 30:1, and 40:1 (v/w, mL/g) were tested in the current work. Several

312 papers reported that varying SSR can influence the extraction of bioactive compounds (Papoutsis et

313 al., 2018). For this reason, in the present research, the extraction has been carried out by varying the

314 SSR and tiny differences have been noticed in the vitamin content extracted using diverse SSR. In

315 detail, SSR of 30:1 and 20:1 seemed to slightly increase the extraction of vitamins.

316 Niacin (2.51−3.07 μg/g), i.e., nicotinic acid (1.88−2.33 μg/g) and nicotinamide (0.63−0.74 μg/g),

317 occurred at higher levels than riboflavin (0.18−0.20 μg/g) in CSS. These results are consistent with

318 those reported in the literature in which niacin occurred at a higher level than riboflavin in coffee

319 beverages (Wachamo, 2017). The levels of niacin especially that of nicotinic acid varies according

320 to the brewing method and roasting profile since it is derived from the second main alkaloids in

321 coffee, trigonelline, which during roasting is degraded into several volatile (pyridine and pyrrole

322 derivatives) and non-volatile compounds (nicotinic acid) (Jeszka-Skowron, Frankowski, & Zgoła-

323 Grześkowiak, 2020). The presence of these two important vitamins, which act as co-factors in

324 numerous essential enzymatic reactions fundamental to maintain the body homeostasis (Thakur et

325 al., 2017), highlights the coffee silverskin value and leads the way for innovative reuses.

326 **3.4 Phytosterol composition of CSS**Phytosterols in CSS

327 The analysis of phytosterols (PS) was performed in CSS using a developed and validated

328 derivatization method allowing the analysis of main sterols in HPLC at higher and more selective

329 detection wavelengths. Few studies assessed the content of PS in CSS. Analyses allowed the

330 identification and quantification of 4 sterols: Δ5-avenasterol, β-sitosterol, campesterol, and

331 stigmasterol for a total concentration of 98.4 ± 8.0 mg/kg (**Table 5**). β-sitosterol was the most

332 abundant sterol (77.1 ± 2.8 mg/kg) followed by campesterol (10.9 ± 3.1 mg/kg), stigmasterol (9.9 ±

333 2.2 mg/kg), and Δ5-avenasterol (0.5 ± 0.1 mg/kg). These results are concordant with the study of

334 Toschi et al (2014). CSS can be considered as a potential source of PS, which are highly valorized

in food and nutraceutical industries due to their hypocholesterolemic effect. Del Pozo et al. (2021) proposed the conversion through pyrolysis of CSS into value-added products. The obtained pyrolysis liquids were rich in caffeine, polyphenols, and β-sitosterol. CSS could thus be transformed into a PS-enriched ingredient with high value-added applications. However, the high temperatures reached during coffee roasting (170–260 °C), lead to the oxidation of PS in CSS. Indeed, PS are susceptible to oxidation at high temperatures forming phytosterol oxidation products (POPs) including 7-hydroxysterols, 7-ketosterols, 5,6-epoxysterols, and 3,5,6-triols, which are reported to possess pro-atherogenic and pro-inflammatory properties (Kamgang Nzekoue et al., 2020). In CSS, Toschi et al (2014) reported considerable levels of POPs with an oxidation rate between 27.6 % and 48.1 %. Therefore, the valorization of CSS for PS production will necessarily require the prior removal of POPs using separation techniques such as solid-phase extraction (Azadmard-Damirchi & Dutta, 2009).

3.5 4. Discussion

Consistent amounts of by-products are generated during the coffee industrial processing and according to FAO, growth projections on coffee production and consumption are expected to increase over the next years. In 2019 the world production accounted for 10,035,576 tonnes with a harvested area of 11,120,498 hectares, and data compared to 2009 respectively account for + 1.5% in production and + 6.31% in the harvesting surface (FAO, 2019). Wastes generated from the coffee industries should be considered useful resource and some of them have already become a resource, especially in agricultural contexts. For example, spent coffee grounds are used with a «Zero Wastes» approach with different applications in agriculture, such as fertilizer for growing mushrooms, as they contain minerals and nutrients that are useful for their growth. Other applications contemplate the use of coffee wastes to create pellet or to produce biobased materials such as pens and cups.

Consideration should be given to the environmental impacts caused by these agricultural by-products, especially in regions dedicated to its cultivation and processing, such as in Latin America

361   (Iriondo-DeHond, et al., 2017). Moreover, in developing countries, such as Uganda, the importance

362   of the coffee sector is relevant as a key driver of rural economic activity and income sources

363   (Mwesigye & Nguyen, 2020). Therefore, a sustainable and fair coffee value chain is a key element

364   in the valorization of coffee wastes in a Circular Economy approach in order to cope with

365   environmental issues and market instability.

366   CSS presents the advantage to be easily collected in high quantity due to its production in coffee-

367   roasting industries. Moreover, CSS is a more stable material, with a low moisture content (ranging

368   between 5 and 10%) and a lower microbial charge due to the high temperatures reached during

369   roasting (Bessada et al., 2018). Compared to other coffee by-products like spent coffee ground, CSS

370   is thus easily exploitable and more advantageous for industrial transformations.

371   The present research highlighted significant levels of healthy nutrients and bioactive compounds in

372   CSS (proteins, minerals, dietary fibers, …), which can thus be used in the development of

373   functional ingredients for food and pharmaceutical uses. Attention should be given to the

374   antinutritional factors and toxic compounds revealed upper (POPs, heavy metals), which have to be

375   removed before potential valorization as food ingredients.

376   Moreover, this study assessed and reported for the first time the levels of B-vitamins (B_2 and B_3) in

377   CSS confirming the initial hypothesis. The increase of knowledge on CSS will speed up the design

378   of novel value-added applications for CSS. The adoption of certified sustainable production of

379   coffee is estimated to bring positive social impacts while implying increased farm incomes with

380   more stable employment possibilities for farmworkers, thus enhancing community development as

381   well as reducing environmental and health risks caused by the accumulation of coffee wastes (Word

382   Bank, 2019).

383   **4.5. Conclusions**

384   The growing research of value-added applications for CSS valorization is nowadays a priority

385   research line to face the environmental challenges of the coffee production system and by-products

386   accumulation. CSS valorization requires analytical studies, in order to quantify healthy nutrients

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387 and bioactive compounds, which could be extracted to develop functional ingredients with potential

388 applications in the food, pharmaceutical, or cosmetic sectors. In this study, the profile of the less

389 studied compounds in CSS including microminerals, phytosterols, and vitamins B_2 and B_3 was

390 assessed and reported. The novelty accompanying the obtained results, confirms the under-

391 exploration of CSS potentialities. Further studies are thus required for a comprehensive evaluation

392 of CSS properties to increase the sustainability of the coffee agro-industry.

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Highlights

1- Nutritional composition of coffee silverskin (CSS) from arabica coffee.

2- Comprehensive assessment of macrominerals (7) and microminerals (23) in CSS.

3- Vitamin B_3 (2.51−3.07 µg/g) and vitamin B_2 (0.18−0.20 µg/g) are present in CSS.

4- Phytosterols analysis revealed high levels of β-sitosterol (77.1 ± 2.8 mg/kg).

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522   **Highlights**

Compound	Retention time (Rt) (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Polarity
Nicotinic acid	1.6	124	80[a]	92	21	positive
			53		33	
Nicotinamide	1.7	123	80[a]	92	21	positive
			53		33	
Riboflavin	2.1	377	243[a]	131	21	positive
			172		41	

523   5- Nutritional composition of coffee silverskin (CSS) from arabica coffee.

524   6- Comprehensive assessment of macrominerals (7) and microminerals (23) in CSS.

525   7- Vitamin B_3 (2.51−3.07 µg/g) and vitamin B_2 (0.18−0.20 µg/g) are present in CSS.

526   8- Phytosterols analysis revealed high levels of β-sitosterol (77.1 ± 2.8 mg/kg).

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528   **Table 1.** HPLC-MS/MS acquisition parameters used for the quantification of niacin (nicotinic acid

529   and nicotinamide) and riboflavin

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533   [a] These product ions were used for quantification, the other for confirming the analytes.

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537 **Table 1.** HPLC-MS/MS acquisition parameters used for the quantification of niacin (nicotinic acid

538 and   nicotinamide)

540 and riboflavin

Compounds	Unit	Levels[a]

541

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544 [a] These product ions were used for quantification, the other for confirming the analytes.

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Compound	Concentration range (ng/mL)	Regression equation	R^2	LOD (ng/mL)	LOQ (ng/mL)	Intraday repeatability (RSD%)	Interda repeatabi (RSD%
Nicotinic acid	3–5000	y = 17500x + 1154.1	0.9971	1	3	2.5	4.2
Nicotinamide	3–5000	y = 25894x + 2369.6	0.9953	1	3	2.7	3.9
Riboflavin	3–5000	y = 11855x + 1163	0.9945	1	3	4.6	6.2

548 **Table 23.** HPLC-MS/MS validation parameters such as linearity, sensitivity, repeatability, and
549 recovery

550 44.

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Nicotinamide	3–5000	y = 25894x + 2369.6	0.9953	1	3	2.7	3.9
Riboflavin	3–5000	y = 11855x + 1163	0.9945	1	3	4.6	6.2

551 **Table 23.** HPLC-MS/MS validation parameters such as linearity, sensitivity, repeatability, and
552 recovery

553 45.

554 **Table 32.** Nutritional composition and minerals profile of coffee silverskin

23

Nutrients

Ash	g/100 g	4.9 ± 0.1
Carbohydrates	g/100 g	18.0 ± 3.0
Fats	g/100 g	3.7 ± 0.3
Moisture	g/100 g	7.7 ± 0.5
Proteins	g/100 g	14.2 ± 1.0
Total dietary fibers	g/100 g	51.5 ± 9.7

Macrominerals

Calcium	mg/100 g	1080 ± 69
Chloride	mg/kg	12.3 ± 0.4
Magnesium	mg/100 g	257 ± 18
Phosphorus	mg/100 g	12.4 ± 0.8
Potassium	mg/100 g	972 ± 46
Sodium	mg/100 g	11 ± 1
Sulphur	mg/kg	51.9 ± 1.3

Microminerals

Aluminum	mg/kg	89.0 ± 6.5
Antimony	mg/kg	0.05 ± 0.009
Arsenic	mg/kg	0.1 ± 0.04
Barium	mg/kg	66.75 ± 12.4
Beryllium	mg/kg	≤0.01
Boron	mg/kg	26.1 ± 4.8
Cadmium	mg/kg	0.07 ± 0.01
Chrome	mg/kg	0.23 ± 0.02
Cobalt	mg/kg	0.2 ± 0.06
Copper	mg/kg	37.9 ± 9.7
Iron	mg/kg	238 ± 11
Lead	mg/kg	0.3 ± 0.005
Manganese	mg/kg	46.7 ± 2.8
Mercury	mg/kg	0.05 ± 0.002
Molybdenum	mg/kg	0.2 ± 0.04
Nickel	mg/kg	0.5 ± 0.01

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Compounds	Unit	Levels[a]
Selenium	mg/kg	0.1 ± 0.03
Silver	mg/kg	0.03 ± 0.006
Thallium	mg/kg	< 0.01
Tin	mg/kg	0.05 ± 0.004

Table 3. Nutritional composition and minerals profile of coffee silverskin

Compounds	Unit	Levels[a]
Nutrients		
Ash	g/100 g	4.9 ± 0.1
Carbohydrates	g/100 g	18.0 ± 3.0
Fats	g/100 g	3.7 ± 0.3
Moisture	g/100 g	7.7 ± 0.5
Proteins	g/100 g	14.2 ± 1.0
Total dietary fibers	g/100 g	51.5 ± 9.7
Macrominerals		
Calcium	mg/100 g	1080 ± 69
Chloride	mg/kg	12.3 ± 0.4
Magnesium	mg/100 g	257 ± 18
Phosphorus	mg/100 g	12.4 ± 0.8
Potassium	mg/100 g	972 ± 46
Sodium	mg/100 g	11 ± 1
Sulphur	mg/kg	51.9 ± 1.3
Microminerals		
Aluminum	mg/kg	89.0 ± 6.5
Antimony	mg/kg	0.05 ± 0.009
Arsenic	mg/kg	0.1 ± 0.04
Barium	mg/kg	66.75 ± 12.4

[a] All the experiments were carried out in three replicates (n=3) and the results are expressed as mean ± standard deviation (SD).

25

Beryllium	mg/kg	≤ 0.01
Boron	mg/kg	26.1 ± 4.8
Cadmium	mg/kg	0.07 ± 0.01
Chrome	mg/kg	0.23 ± 0.02
Cobalt	mg/kg	0.2 ± 0.06
Copper	mg/kg	37.9 ± 9.7
Iron	mg/kg	238 ± 11
Lead	mg/kg	0.3 ± 0.005
Manganese	mg/kg	46.7 ± 2.8
Mercury	mg/kg	0.05 ± 0.002
Molybdenum	mg/kg	0.2 ± 0.04
Nickel	mg/kg	0.5 ± 0.01
Selenium	mg/kg	0.1 ± 0.03
Silver	mg/kg	0.03 ± 0.006
Thallium	mg/kg	≤ 0.01
Tin	mg/kg	0.05 ± 0.004
Titanium	mg/kg	2.1 ± 0.1
Vanadium	mg/kg	0.2 ± 0.06
Zinc	mg/kg	31.9 ± 5.3

[a] All the experiments were carried out in three replicates ($n=3$) and the results are expressed as mean ± standard deviation (SD).

Table 4. Content (µg/g) of niacin and riboflavin in coffee silverskin using different solvent-to-sample ratios (SSR), i.e., 20:1, 30:1 and 40:1

	SSR[a] (mL/g)		
Compounds	20:1	30:1	40:1
Niacin (B$_3$)	2.92 ± 0.10	3.07 ± 0.13	2.51 ± 0.16
- Nicotinamide	0.70 ± 0.06	0.74 ± 0.06	0.63 ± 0.02
- Nicotinic acid	2.22 ± 0.03	2.33 ± 0.07	1.88 ± 0.13
Riboflavin (B$_2$)	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.01

[a] All the experiments were carried out in three replicates ($n=3$) and the results are expressed as mean ± standard deviation (SD).

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Table 4. Content (µg/g) of niacin and riboflavin in coffee silverskin using different solvent-to-sample ratios (SSR), i.e., 20:1, 30:1 and 40:1

Compounds	Unit	Levels[a]
Campesterol	mg/kg	10.9 ± 2.1
Stigmasterol	mg/kg	9.9 ± 1.6
β-Sitosterol	mg/kg	77.1 ± 2.8
Δ5-Avenasterol	mg/kg	0.5 ± 0.1

Compounds	SSR[a] (mL/g)		
	20:1	30:1	40:1
Niacin (B_3)	2.92 ± 0.10	3.07 ± 0.13	2.51 ± 0.16
- Nicotinamide	0.70 ± 0.06	0.74 ± 0.06	0.63 ± 0.02
- Nicotinic acid	2.22 ± 0.03	2.33 ± 0.07	1.88 ± 0.13
Riboflavin (B_2)	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.01

[a] All the experiments were carried out in three replicates (n=3) and the results are expressed as mean ± standard deviation (SD).

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Table 5. Concentrations of phytosterols in coffee silverskin

Compounds	Unit	Levels[a]
Campesterol	mg/kg	10.9 ± 2.1
Stigmasterol	mg/kg	9.9 ± 1.6
β-Sitosterol	mg/kg	77.1 ± 2.8
Δ5-Avenasterol	mg/kg	0.5 ± 0.1

[a] All the experiments were carried out in three replicates ($n=3$) and the results are expressed as mean ± standard deviation (SD).

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Table 5. Concentrations of phytosterols in coffee silverskin

[a] All the experiments were carried out in three replicates ($n=3$) and the results are expressed as mean ± standard deviation (SD).

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