Towards Coffee Processing by-Products Valorization: Phenolic Compounds and Antioxidant Activity of Spent Coffee Grounds and Coffee Silverskin

Mariem Saada[#1], Mariam Kasmi[*23], Riadh Ksouri[#1]

[#1] *Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cédria (CBBC), BP 901, 2050 Hammam-lif, Tunisia.*

[#2]*High School of Food Industry of Tunis (ESIAT), University of Carthage, Cité El Khadhra, Tunisia.*

[#3] *Laboratoire de Traitement et de Valorisation des Rejets Hydriques (LTVRH), Water Researches and Technologies Center (CERTE), University of Carthage, Tourist route Soliman, BP 273-8020 Nabeul, Tunisia*

Abstract

Coffee processing byproducts are studied in this work for their valuable components. Spent coffee grounds (SCG) and coffee silverskin (CSS) from Coffea arabica species were investigated for the determination of ethanol/water extracts bioactive molecules of interest. Phenolic compounds evaluation revealed that SCG enclose important amounts of polyphenols (8.425 mg GAE.g^{-1} DW) and flavonoids (2.801 mg CE.g^{-1} DW). Nevertheless, the highest levels of tannin (3.45 mg CE.g^{-1} DW) were obtained with CSS. The total antioxidant activity of SCG was equal to 8.267 mg GAE.g^{-1} DW, the DPPH test showed an IC_{50} value of 18 µg.ml^{-1} and the inhibition of β-carotene bleaching of IC_{50} was 600 µg.ml^{-1}. While, the CSS showed better reducing power with an IC_{50} value of 120 µg.ml^{-1}. Therefore, SCG proved to be more interesting than CSS regarding its antioxidant potentiality and its richness in phenolic compounds to be incorporated as food or therapeutic additive.

Keywords

Spent coffee grounds, Coffee silverskin, Valorization, Phenolic compounds, Antioxidant activity.

I. INTRODUCTION

Nowadays, the fundamental research is focusing an increasing interest for plants composition, uses and identification of bioactive compounds for more effective foods and remedies production [1]. In both therapy and food industries, plants biological activities have been proven to be stronger than synthetic antioxidants. Furthermore, synthetic antioxidants are blamed for their undesirable effect on human health [2]. On another hand, relevant plant derived investigations have been expanded due to the commercial application demand of the bioactive molecules for cosmetic uses [3]. Several natural antioxidants selections are used as food additives for food quality preservation by slowing down degradation mechanisms [4].

Coffea is a genus of flowering plants in the family *Rubiaceaea*. The plant seeds are known as coffee beans and they are used to flavor several beverages and products. Many beneficial effects of coffee enclosing the antioxidant activity can be ascribed to its bioactive molecules, namely the phenolic compounds. Nevertheless, during the coffee beans industrial processing to produce instant coffee, large amounts of by-products are generated. Disposal of these by-products are currently an environmental concern [5]. Accordingly, efforts need to be made for the reuse and the valorization of those by-products. The coffee bean accounts for approximately 50% of the coffee cherry dry weight and produces spent coffee grounds (SCG) as the final by-product of the coffee industry. During the solubilized instant coffee production, coffee beans are roasted, ground and then heat or steam treated. The produced coffee extract is dedicated for consumption and the remaining residue is generated as SCG. In the world, six million tons of SCG are nearly produced per year [6]. The second by-product is the integument of the coffee bean produced during roasting and it is referred to coffee silver skin (CSS). The CSS contains important amounts of total dietary fiber, antioxidant activity and phenolic compounds although it accounts for only 1–2% of the total coffee berry [7].

By reference to the International Coffee Organization statistical data, the annual coffee production increased from 8400 to 9120 thousand tons since 2010 [8]. Thus, the smart handling of coffee by-products became a serious challenge. Utilizing these by-products as a base or substrate for value adding applications is an effective way to minimize their wastage as landfill [9]. In the frame of coffee byproducts valorization and reuse, SCG and CSS from a Tunisian coffee industry were evaluated for their phenolic compounds and antioxidant activities in the aim to their

incorporation as food additives or pharmaceutical substances.

II. MATERIALS AND METHODS

A. Samples collection and preparation

The studied coffee by-products in this paper belong to *Coffea arabica* species. The coffee silver skin (CSS) also called the perisperm or spermoderm and the spent coffee grounds (SCG) were collected from the coffee company "ORO" located in the industrial zone of Charguia I in Tunisia. Samples were immediately returned to the laboratory to be dried at 60°C for 24 hours in an oven. The silver skin samples were then finely ground using a ball mill (Dongoumeau type). The powder was stored in the dark and at 4°C. Different extractions with ethanol / water at different percentages were performed on both samples. A mass of 2 g of dry matter was added to 20 ml of the ethanol/water mixture by varying the percentage (pure ethanol, 80, 50, 20% (ethanol/water) and pure water). The mixtures were kept for 24 h at 4°C and filtered through Whatman No. 4 filter paper. Extracts were stored at 4°C until analysis.

B. Colorimetric quantification of phenolics

Total phenolic content were assayed by the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. [10]. Total flavonoids were measured according to the protocol proposed by Zhishen et al. [11]. Procyanidins were measured using the modified vanillin assay described by Sun et al. [12]. The detailed experimental protocol is described by Saada et al. [13].

C. Assessment of antioxidant activities

1) ***Evaluation of total antioxidant capacity (TAC)***: The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH [14]. The detailed experimental protocol is described by Saada et al. [13]

2) ***DPPH radical–scavenging activity***

The di-phenyl-picryl-hydrazine (DPPH) quenching ability of organ extracts was measured according to the method described by Hatano et al. [15]. The detailed experimental protocol is described by Saada et al. [13]. Each fraction was analyzed in triplicate.

3) ***Determination of the ferric antioxidant reducing power (FRAP)***

The method of Oyaizu [16] was used to assess the reducing power of shoot. Methanol extracts (0.2 ml) were prepared at different concentrations (from 100 to 1000 µg.ml-1 for the CSS extract and from 100 to 500 µg.ml-1 for CSG extract). Samples were mixed with 0.5 ml of sodium phosphate buffer (pH = 6.6, 0.2 M) and 0.5 ml of potassium ferrocyanide $K_3Fe(CN)_6$ (1%) and incubated in a water bath at 50°C for 20 min. Then, 0.5 ml of trichloroacetic acid (10%) was added and the mixture was centrifuged at 650 × g for 10 min. The supernatant (0.5 ml) was then mixed with an equal volume of distilled water and 0.1 ml of ferric chloride (0.1%). The intensity of the blue-green appearing color was measured at 700 nm. The IC_{50} value (µg ml^{-1}) for the reducing power is the extract concentration at which the absorbance was 0.5. The ascorbic acid was used as a positive control.

4) ***β-Carotene bleaching test (BCBT)***

Analysis were performed according to the method described by Koleva et al. [17]. The detailed experimental protocol is described by Saada et al. [13]

D. Statistical analysis

Results were analyzed statically by the one-way analysis of variance (ANOVA) methodology. The test of Student-Newman-Keuls was applied to determine the least significant differences (LSD) among means at $p < 0.05$ using IBM® SPSS version 20.0.0 software.level-1 heading mTotal phenolic content were assayed by the Folin-Ciocalteu

III. RESULTS AND DISCUSSION

A. Screening of coffee by-products extracts: Comparative Test

The extraction experiments were performed using different conditions of ethanol concentration (0–100%), solvent/solid ratio of 10 ml/g and extraction time of 24h. Therefore, this study consisted in evaluating the effect of the solvent concentration on the recovery of antioxidant phenolic compounds from SCG and CSS by-products.

1) ***Total phenolic content***

The comparison between the different ethanol/water extracts of the coffee wastes (CSS and SCG) showed a wide range of phenolic compounds contents. Statistical analysis of these data revealed a significant influence ($p < 0.05$) of all the studied variables on the extraction results. In fact, the total phenol contents vary from 1.97 (pure ethanol extract) to (50% ethanol extract) 6.3 mg GAE.g^{-1} DW for CSS by-product. The latter thus presented the best extracting power followed by the 80% ethanol extract and the 20% ethanol extract (Fig. 1). The SCG analysed extracts maintain the same order in terms of their phenolic compounds contents. The recorded values ranged from 1.4 mg GAE.g^{-1} DW for pure ethanol extract to 8.4 mg GAE.g^{-1} DW for 50% ethanol extract. By this way, the high phenolic compounds extraction was obtained with the 50% ethanol extracts for both CSS and SCG by-products. Nevertheless, it is worthy to note that the 50% ethanol extract of the SCG samples contains 33% more total polyphenols than the CSS ones. These findings are in accordance with the recent investigation performed by Regazzoni et al. [18] showed that the total polyphenol contents were

12.29 mg GAE.g^{-1} DW of SCG and 10.75 mg GAE.g^{-1} DW of CSS using 70% ethanol extractions in a solvent/solid ratio of 10 ml/g during 18h for *Coffea arabica* species. In addition, Mussatto et al. [5] reported that extraction using 60% methanol in a solvent/solid ratio of 40 ml/g SCG during only 90 min was the most suitable condition to produce an SCG extract with high content of phenolic compounds (16 mg GAE.g^{-1} DW SCG) and high antioxidant activity (Ferric reducing antioxidant power of 0.10 mM Fe(II).g^{-1}), simultaneously .

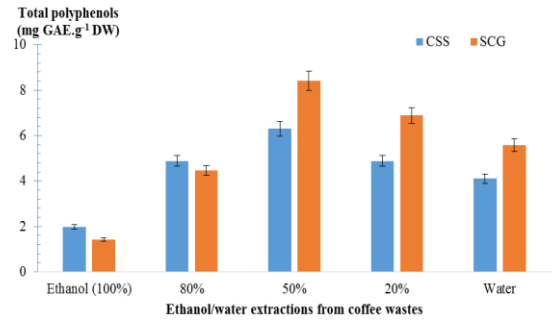

Fig.1 Total polyphenols contents distribution according to the ethanol/water extractions of coffee skin silver (CSS) and spent coffee grounds (SCG) wastes

2) ***DPPH radical–scavenging activity***

The radical scavenging activity was determined by DPPH for all the ethanol/water extracts of both SCG and CSS samples (Fig 2). Both 20 and 50% ethanol extracts recorded interesting IC_{50} values for SCG by-product (20 and 18.5 µg.ml^{-1}, respectively). However, the pure ethanol extract recorded the most interesting IC50 value for CSS samples (44 µg.ml^{-1}). The antioxidant activity of the SCG 50% ethanol extract (18.5 µg.ml^{-1}) was found quite comparable to that of the synthetic antioxidant standard (BHT) 11.5 µg.ml^{-1}. Meanwhile, the SCG and CSS of the Coffee Arabica species studied by Regazzoni et al. [18] revealed IC_{50} values of 10.59 and 11.26 µg.ml^{-1}, respectively; which are much closer even better than the synthetic antioxidant value.

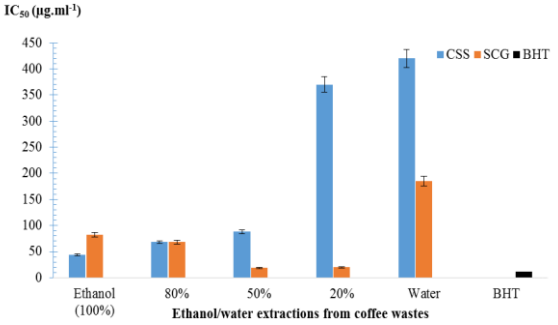

Fig.2 DPPH radical-scavenging activity expressed in CI_{50} (µg.ml^{-1}) distribution according to the ethanol/water extractions of coffee skin silver (CSS) and spent coffee grounds (SCG) wastes compared to the synthetic antioxidant (BHT) as a control.

B. The selected extracts characterization: Phenolic and Antioxidant compounds

1) ***Phenolic compounds: Total polyphenols, flavonoids and tannins***

The phenolic compound contents of the waste extracts revealed significant differences (Table 1). SCG showed important total polyphenols amounts (8.4 mg GAE.g^{-1} DW) compared to the CSS contents (1.975 mg GAE.g^{-1} DW). By reference to the coffee beans contents of both *Arabica* and *Robusta* varieties investigated in previous works, the studied coffee by-products enclosed 15% of the coffee beans polyphenol contents [19]. Nevertheless, it is important to pinpoint that the SCG 50% ethanol extract in this work contains higher phenolic compounds compared to some fruits, such as grapes (7 mg GAE.g^{-1} DW) [20] and Mango (6.25 mg GAE.g^{-1} DW) [21]. As for the condensed tannins contents, CSS pure ethanol extract exhibited more interesting amount (3.8 mg CE.g^{-1} DW) compared to the SCG content (0.997 mg CE.g^{-1} DW).

Table 1. Phenolic compounds and antioxidant activities of the CSS ethanol (100%) extract and the SCG ethanol (50%) extract.

	Units	CSS (100%)	SCG (50%)
Total polyphenols	mg GAE/g DW	1.97 ±0.12^{a}	8.42 ±0.77^{b}
Condensed tanins	mg CE/g DW	3.84 ±0.30^{a}	0.997 ±0.1^{b}
Flavonoids	mg CE/g DW	2.58 ±0.67^{a}	2.8 ±0.81^{a}
Total antioxydant activity (TAA)	mg GAE/g DW	7.00±0.92^{a}	8.26 ±1.21^{a}
Iron reducing activity	µg/ml	120 ±2^{a}	440 ±2^{b}
IC_{50} (DPPH)	µg/ml	44 ±1.4^{a}	18.5 ±1.6^{b}
β-carotene	µg/ml	1700 ±2.7^{a}	600 ±2.3^{b}

^{a,b} Statistical differences within a line are shown with different lowercase letters

The condensed tannins content of the CSS pure ethanol extract is as interesting as the *C. arabica* extract content estimated at 3.97 mg CE.g-1 DW by Clifford and Ramirez-Martinez [21]. But, both of them remain far from the condensed tannins content of the variety *C. robusta* estimated at 10.9 mg CE.g-1 DW) by the same authors. However, the studied SCG extract flavonoids content (2.8 mg CE.g^{-1} DW) is slightly greater than CSS extract content (2.5 mg CE.g^{-1} DW). Those values are comparable to the SCG flavonoid contents ranging from 0.51 to 2.5 mg CE.g-1 DW [5]. Nevertheless, black and green tea seem to be of exceptional flavonoid richness where the estimated amounts exceeded 140.7 mg CE.g^{-1} DW [23].

2) ***Antioxidant capacity: Total antioxidant activity, Iron reducing power and β-carotene bleaching inhibition***

Antioxidant capacity parameters assessment results of SCG and CSS by-products are shown in the Table 1. The TAA values exhibited no significant difference ($p < 0.05$) between SCG 50% ethanol extract and CSS pure ethanol extract. However, for the antiradical activity DPPH, the iron reducing power and β-carotene bleaching test significant differences were detected between the coffee by-products extracts. In fact, the antiradical activity against the radical DPPH was more important for SCG extract (IC_{50} = 18 µg.ml^{-1}) compared to the recorded value with CSS extract (IC_{50} = 44 µg. ml^{-1}). By reference to previous works, it has been reported that *C. arabica* IC_{50} values ranged from 16.11 to 24.92 µg.ml^{-1} and *C. robusta* values ranged from 14.70 to 19.47 µg.ml^{-1} [24]. By this way, it could be concluded that SCG extract exhibited comparable antiradical activity to the coffee bean one. On the other hand, the reaction between the phenolic antioxidants and the DPPH radical is dependent on the structural conformation of these antioxidants [25]. Indeed, some compounds react very quickly with the DPPH by reducing it in equal number as the hydroxyl groups [26]. Currently, reducing power is an important parameter for estimating antioxidant activity [27]. Comparison of the two by-products of coffee revealed that CSS has a greater ferric ion reduction capacity than SCG (120 and 440 µg.ml^{-1} for CSS and SCG extracts respectively). Besides, the extract of coffee grounds has shown more interesting ability to inhibit the bleaching of β-carotene (600 µg.ml^{-1}) than the coffee skin extract (1700 µg.ml^{-1}). According to Liyana-Pathirana et al. [27], an extract that delays or inhibits the bleaching of β-carotene can be described as a free radical scavenger and a primary antioxidant. In fact, the inhibition test of linoleic acid oxidation coupled with that of β-carotene appears to be very useful as a mimetic model of lipid peroxidation in biological membranes [28, 29].

The important difference between the two studied SCG and CSS coffee by-products on the basis of their antioxidant activities and their contents in phenolic compounds, indicate that these activities do not depend only on the concentration of phenolic compounds but also on the structure and the interaction between the different compounds. These differences between suggest that each test has a different mechanism of action that depends on the different phytochemicals [30]. Finally, based on the polyphenol content and the antioxidant activities, both SCG and CSS byproducts could be considered as a source of antioxidants. It is therefore interesting to extend the exploration of their richness in phenolic compounds and other natural antioxidants.

IV. CONCLUSIONS

Spent coffee grounds (SCG) and coffee silverskin (CSS) byproducts from *Arabica* coffee were investigated for their bioactive molecules. Total polyphenols content and the anti-radical activity against the DPPH radical for different ethanol/water extracts showed that using pure ethanol was the more efficient for the CSS. Whereas, SCG extract using 50 % ethanol as solvent exhibited the most interesting results. Important amounts of polyphenols (8.425 mg GAE.g^{-1} DW) and flavonoids (2.8 mg CE.g^{-1} DW) were detected with SCG. Nevertheless, the highest levels of tannin (3.45 mg CE.g^{-1} DW) were obtained with CSS. In addition, SCG by-product recorded a TAA of about 8.26 mg GAE.g^{-1} DW, an IC_{50} value of 18 µg.ml^{-1} and an inhibition of bleaching of β-carotene value of 600 µg.ml-1. However, the CSS showed better reducing power with an IC_{50} value of 120 µg.ml^{-1}. Those findings proved that both SCG and CSS by-products present interesting proprieties regarding their antioxidant potentiality and their richness in phenolic compounds. As a result, the industrial utility of such by-products as antioxidant adjuncts for food processing should be implemented in further investigations.

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