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Bioactive Compound Content and Antioxidant Capacity of Solid Processing Waste from Organic and Conventional Coffee Farming

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ABSTRACT Brewed coffee is a rich source of natural antioxidants and other bioactive compounds. The coffee industry generates high volumes of solid by-products, both during the coffee berry processing (pulp and silver skin) and the actual brewing (spent coffee grounds). These may contain important levels of bioactive compounds but are usually not exploited and treated as waste. On the other hand, farming methods may influence the quality of crops. Unlike conventional farming methods, the production of organic foods may contribute to enhancing their bioactive compound content while protecting the environment. The aim of this work was to determine the bioactive compound content in dried coffee processing waste. The effect of farming method (organic and conventional) on total phenolic and flavonoid compound content and the antioxidant capacity was studied in coffee silver skin and spent coffee grounds. The Arabica coffee whose processing by-products were used in this work proceeded from Ixhuatlán del Café (Veracruz,

Mexico). The samples were dried at 65°C to constant weight. Total phenolic and flavonoid content and the antioxidant capacity of the samples were determined. Samples from organic farming presented higher bioactive compound content and antioxidant capacity than samples from conventional farming. For example, total phenolic compound content in silver skin samples was significantly (p<0.05) higher in organic samples (2.6±0.7 mg GAE/g DB) compared to the conventional ones (0.7±0.1 mg GAE/g DB). These results suggest that coffee processing by-products might be an interesting source of bioactive compounds, especially when the coffee proceeds from organic farming.

Keywords: Coffee silver skin, spent coffee grounds, phenolic compounds, flavonoids, coffee by-products.

INTRODUCTION Brewed coffee, a popular beverage consumed world-wide, is a rich source of natural antioxidants and other bioactive compounds (Mussatto et al., 2011a; Mussatto et al., 2011b). However, the quality of coffee can be linked to various environmental, genetic, agronomic and agro-industrial factors (Wintgens, 2004). Among these factors is the type of coffee farming method, which can be either conventional or organic.

Mexico is the biggest producer of organic coffee in the world (Paz et al., 2013). Organic coffee farming includes the use of organic manure and biological pest control instead of artificial fertilizers and pesticides characteristic of conventional farming methods (Lyngbaek et al., 2001). Organic coffee farming is environment-friendly and provides the consumer with a product free of pesticides and fertilizers (Grossman, 2003; Van der Vossen, 2005). Thus, it has gained both popularity and importance in recent years (Gómez Cruz et al., 2010; Willer and Lernoud, 2016). Moreover, organic coffee farming helps with important practices of soil conservation in Mexico (Escamilla et al., 2005). However, the effects of farming method on the bioactive compound content and antioxidant capacity of coffee have not been investigated.

On the other hand, green coffee bean (the only commercially interesting product) constitutes only 50-55% of the total dry matter of the ripe coffee cherry (Vincent, 1998). This means that enormous amounts of solid waste are generated both during the coffee berry processing (pulp and silver skin) and the actual brewing (spent coffee grounds). Crucially, these may contain important levels of bioactive compounds with antioxidant capacity but are usually not exploited and treated as waste. When released into the environment, these coffee processing by-products can become a serious problem due to their low biodegradability and high oxygen demands (Pedraza-Beltrán et al., 2012).

Coffee silver skin is a by-product generated during the coffee roasting process. It represents only about 4% of the total weight of the coffee bean (Esquivel and Jiménez, 2012) but can contain high amounts of phenolic compounds (Borrelli et al., 2004) and dietary fiber (Murthy and Naidu, 2012). On the other hand, about 6 million tons of spent coffee grounds are generated world-wide every year as a result of the coffee brewing process (Mussatto et al., 2011b). However, both coffee processing by-products are usually discarded, used as fuel or compost (Menéndez et al., 2007; Saenger et al., 2001; Zuorro and Lavecchia, 2012).

The aim of the present research was to determine the bioactive compound content in coffee processing waste. The effect of farming method (organic and conventional) on total phenolic and flavonoid compound content and the antioxidant capacity was studied in coffee silver skin and spent coffee grounds.

MATERIALS AND METHODS

Raw materials Ground coffee and silver skin samples were obtained from Mexican coffee traders. All coffee proceeded from the different plantations in Ixhuatlán del Café (Veracruz,

Mexico). Both organic and conventionally farmed coffee samples came from a homogenous mixture of coffee produced by various coffee growers.

Sample preparation Spent coffee grounds were prepared by brewing 25 g of ground coffee in a paper filter by the addition of 250 mL of boiling purified water. The filtrate was discarded and the residue was collected as spent coffee grounds. Subsequently, both coffee processing by-products (coffee silver skin and spent coffee grounds) were dried at 65°C in a hot air oven (Riossa Digital, Model HCF-62) until reaching a constant weight (24 h) and stored in amber glass bottles at controlled temperature (-18°C) until the analysis. The moisture content of the samples was determined using AOAC method 934.01 (AOAC, 1997) and dry basis (DB) of the samples was calculated.

Bioactive compound content The total phenolic compound content was determined by the colorimetric method described by Singleton et al. (1999). The mechanical extraction was carried out in a mortar by adding 0.2 g of dry sample into 10 mL of 80% (v/v) methanol and the mixture was shaken for 1 h. The extracts were centrifuged at 14,000 rpm for 5 min. The reaction mixture was prepared by adding 250 µL of extract to 250 µL of Folin-Ciocalteu reagent (dilution factor 1:4) and 2 mL 2% (w/v) of Na_2CO_3. The samples were allowed to rest for 1 h in the dark. The absorbances were read at 765 nm. The total content of phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per g of DB.

The total flavonoid content was determined according to the colorimetric method described by Khanam et al. (2012). The mechanical extraction was carried out in a mortar by adding 0.2 g of dry sample into 10 mL of 80% (v/v) methanol. The mixture was deposited into test tubes and allowed to boil in a water bath for 1 h, stirring the mixtures every 5 min. Then, the mixtures were cooled in ice water for 20 min. The extracts were centrifuged at 14,000 rpm for 5 min. The reaction mixture was prepared by adding 250 µL of extract to 50 µL of $AlCl_3$ (w/v), 50 µL of CH_3CO_2K (w/v), 1 mL of 80% (v/v) methanol and 1 mL of distilled water. The absorbances of the samples were read at 415 nm. The total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of DB.

Antioxidant capacity The antioxidant capacity was determined by ABTS radical inhibition assay, according to the methodology proposed by Re et al. (1999). The ABTS radical was obtained after the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration), incubated in the dark for 16 h at room temperature (±25°C). The resulting ABTS radical was diluted in pure methanol to an absorbance value of 0.700 (±0.020) at 732 nm (maximum absorption wavelength). Filtered samples were diluted with methanol until an inhibition of 20 to 90% occured, as compared to the absorbance of the blank, after adding 20 µL of the sample. At 980 µL, the absorbance was determined at 732 nm. The antioxidant capacity was expressed as percentage of ABTS radical inhibition.

On the other hand, the antioxidant capacity was also determined by DPPH radical inhibition assay, according to the methodology described by Brand-Williams et al. (1995). The absorbance of the 100 µM DPPH radical (3.9 mL) dissolved in 80% methanol was measured at 517 nm. Then, 0.1 mL of the sample or standard was added. The mixture was homogenized carefully and kept in the dark for 30 min. The antioxidant capacity was expressed as percentage of DPPH radical inhibition.

Statistical analysis The statistical analysis was performed in StatGraphics Centurion XVI.I ®. One-way ANOVAs and *post hoc* analyses (Tukey) were carried out.

RESULTS AND DISCUSSION

Silver skin Figure 1 shows the effect of the farming method on the bioactive compound content and antioxidant capacity in coffee silver skin.

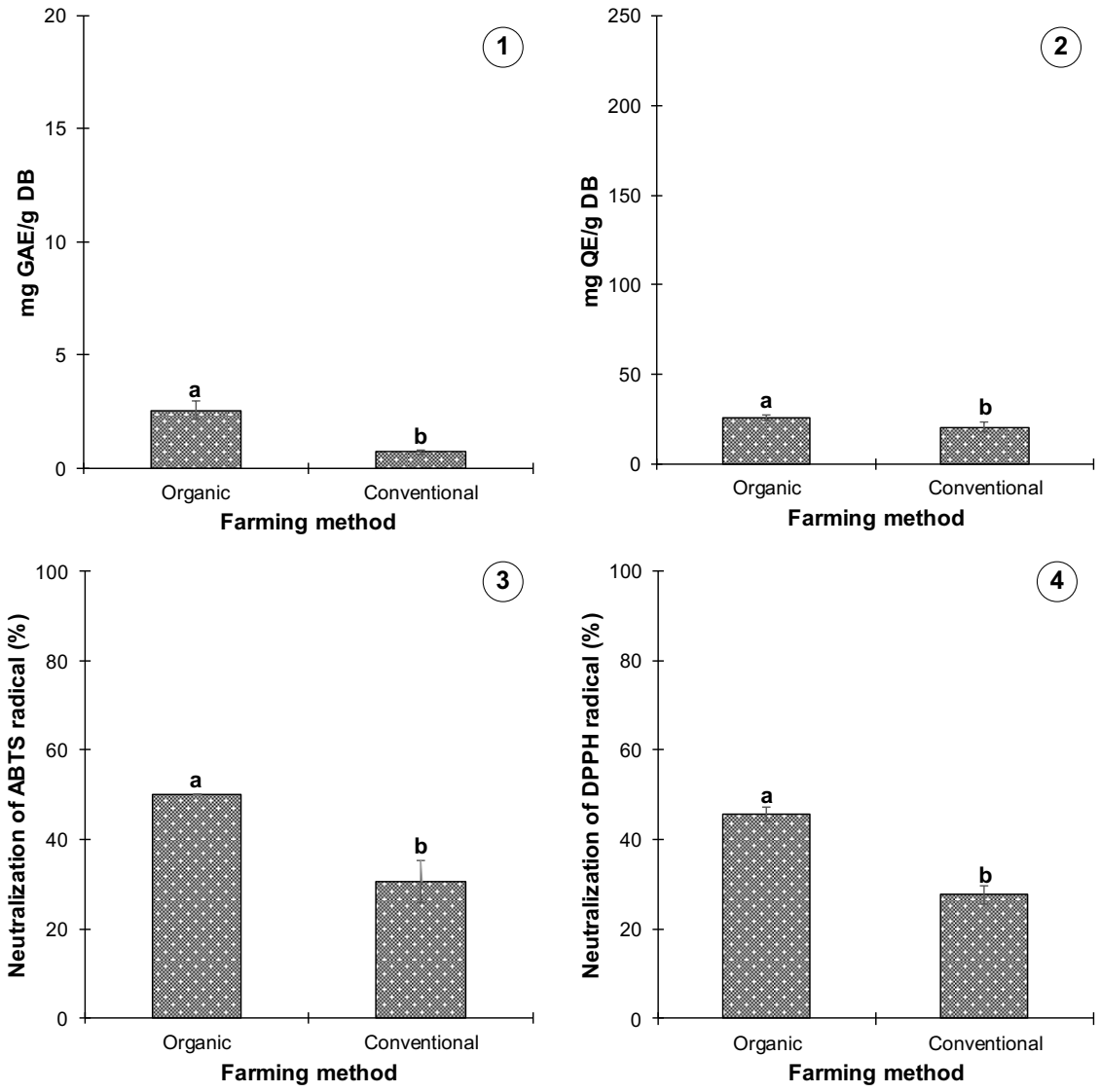

Figure 1. Silver skin**.** The effect of the coffee farming method (organic or conventional) on the concentration of total phenolic compounds (1), total flavonoids (2), the inhibition of ABTS (3) and DPPH (4) radicals in coffee silver skin. The superscripts a and b show significant differences (Tukey, $p \leq 0.05$).

Figure 1.1 shows the content of total phenolic compounds in coffee silver skin from organic and conventional farming. Organic silver skin contained about 3.5-times higher concentration of phenolic compounds (2.55±0.39 mg GAE/g DB) compared to silver skin from conventionally farmed coffee (0.72±0.09 mg GAE/g DB). Previously reported content of total phenolic compounds in coffee silver skin ranges from 2.3 to 36 mg GAE/g DB (Ballesteros et al., 2014; Mussatto, 2015).

Figure 1.2 shows the content of total flavonoid compounds in coffee silver skin from organic and conventional farming. Organic silver skin contained slightly higher concentration of flavonoid compounds (25.70±1.89 mg QE/g DB) compared to silver skin from conventionally farmed coffee

(20.47±2.73 mg QE/g DB). Previously reported content of total flavonoid compounds in coffee silver skin was 1.68±0.06 mg QE/g DB (Ballesteros et al., 2014).

As for the antioxidant capacity, organic silver skin achieved higher percentage of ABTS radical inhibition (50.00±0.00%) than silver skin from conventionally farmed coffee (30.56±4.81%) (Figure 1.3). Organic silver skin also achieved higher percentage of DPPH radical inhibition (45.52±1.56%) than silver skin from conventionally farmed coffee (27.60±1.99%) (Figure 1.4). Previously reported antioxidant capacity (DPPH) of coffee silver skin was 70% (Murthy and Naidu, 2012).

Spent coffee grounds Figure 2 shows the effect of the farming method on the bioactive compound content and antioxidant capacity in spent coffee grounds.

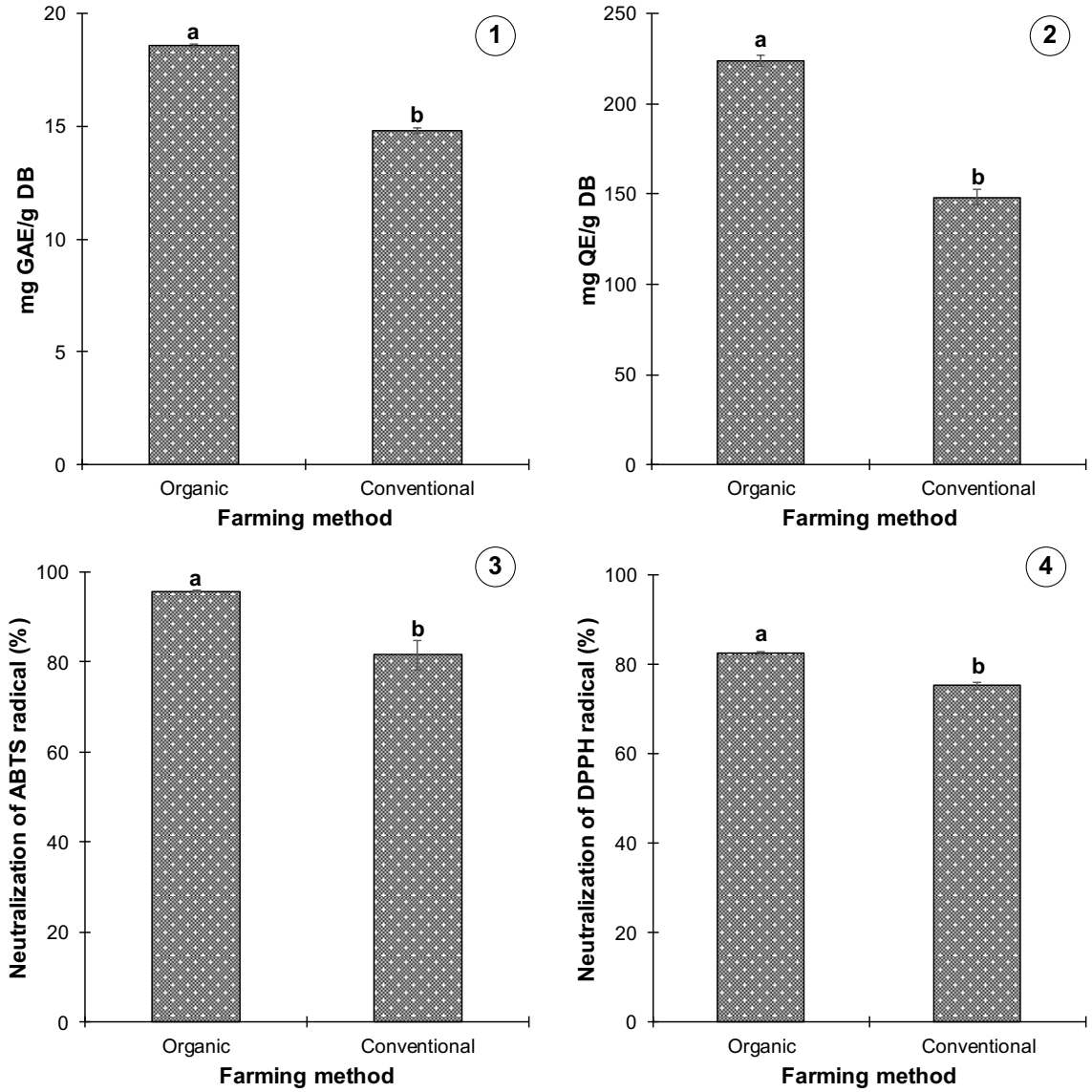

Figure 2. Spent coffee grounds. The effect of the coffee farming method (organic or conventional) on the concentration of total phenolic compounds (1), total flavonoids (2), the inhibition of ABTS (3) and DPPH (4) radicals in spent coffee grounds. The superscripts a and b show significant differences (Tukey, $p \leq 0.05$).

Figure 2.1 shows the content of total phenolic compounds in spent coffee grounds from organic and conventional farming. Organic spent coffee grounds contained slightly higher concentration of phenolic compounds (18.58±0.07 mg GAE/g DB) compared to spent coffee grounds from conventionally farmed coffee (14.81±0.13 mg GAE/g DB). Previously reported content of total phenolic compounds in spent coffee grounds ranges from 2.3 to 28 mg GAE/g DB (Mussatto, 2015, Mussatto et al., 2011a; Sousa et al., 2015).

Figure 2.2 shows the content of total flavonoid compounds in spent coffee grounds from organic and conventional farming. Organic spent coffee grounds contained about 1.5-times higher concentration of flavonoid compounds (224.09±3.05 mg QE/g DB) compared to spent coffee grounds from conventionally farmed coffee (148.19±4.37 mg QE/g DB). Previously reported content of total flavonoid compounds in spent coffee grounds was 1.81 mg QE/g DB (Mussatto et al., 2011a).

As for the antioxidant capacity, organic spent coffee grounds achieved higher percentage of ABTS radical inhibition (95.71±0.10%) than spent coffee grounds from conventionally farmed coffee (81.48±3.21%) (Figure 2.3). Organic spent coffee grounds also achieved higher percentage of DPPH radical inhibition (82.24±0.31 %) than spent coffee grounds from conventionally farmed coffee (75.06±0.70%) (Figure 2.4). Previously reported antioxidant capacity (ABTS) of spent coffee grounds ranges from 5.9 to 22% (Mussatto, 2015).

General discussion Most values reported in this work coincide with values previously reported by other authors, despite the fact that some of them used different extraction techniques. However, the total flavonoid content in both coffee silver skin and spent coffee grounds found in this work is high above the total flavonoid content values previously reported in these coffee by-products by other authors. Nevertheless, chemical composition of plants may vary with different geographic locations, ages of the plant, climate and soil conditions, etc. (Murthy and Naidu, 2012).

In general, spent coffee grounds analyzed in this work showed higher bioactive compound content and antioxidant capacity than coffee silver skin. However, coffee silver skin achieved relatively high values of antioxidant capacity when compared to its bioactive compound content. This could be attributed to the fact that total phenols and flavonoids are not the only compounds responsible for the antioxidant capacity of coffee by-products. Coffee silver skin is a by-product of coffee roasting and during this process, other antioxidants (such as melanoidins) can be synthesized (Borrelli et al., 2004).

As for the effect of the farming method on bioactive compound content and antioxidant capacity of coffee silver skin and spent coffee grounds, organic coffee by-products studied in this work seem to be significantly richer in bioactive compounds when compared to those from conventionally farmed coffee. Up to date, no research has been conducted on the effects of organic and conventional farming method on bioactive compound content and antioxidant capacity of coffee by-products. However, previous research on other plants points to a significant effect of organic farming on the quality of the product. Significantly higher bioactive compound content (polyphenols and flavonoids) has been reported in organic medicinal plants in comparison to those from a conventional farming method (Kazimierczak et al., 2014).

Brandt and Mølgaard (2001), reviewed the effects of organic farming methods on the nutritional quality of foods. Interestingly, the authors suggested that organic vegetables can contain 10-50% more defense-related secondary metabolites (including polyphenols and flavonoids) than conventionally grown vegetables. Indeed, the benefits that organic farming can bring about seem to be related not to the adverse effects of pesticides on human health but to the higher amounts of bioactive compounds that organic plants seem to synthesize in order to defend themselves from the adverse effects of the environment (Colquehuanca and Edy, 2016).

CONCLUSION The results obtained in this work point to possible a beneficial effect of organic farming on bioactive compound content and antioxidant capacity of coffee industry by-products, particularly organic coffee silver skin and spent coffee grounds. Given the fact that Mexico is the biggest producer of organic coffee in the world, solid waste from organic coffee processing should be exploited for the extraction of bioactive compounds or processed and incorporated in foods as functional ingredients with antioxidant capacity. However, bioactive compound extracts from organic coffee by-products need to be carefully characterized and their components identified before any implementation can take place. Therefore, a lot of research is needed in this area of opportunity.

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