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Characterization of a New Potential Functional Ingredient: Coffee Silverskin

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Dietary fiber (DF) is one of the main dietary factors contributing to consumers' well-being. In this work the possibility of using the roasted coffee silverskin (CS), a byproduct of roasted coffee beans, as a DF-rich ingredient has been evaluated. The results of our investigation showed that this material has 60% total DF, with a relevant component (14%) of soluble DF. Although a small amount of free phenol compounds is present in CS, it has a marked antioxidative activity, which can be attributed to the huge amount of Maillard reaction products, the melanoidins. Static batch culture fermentation experiments showed that CS induces preferential growth of bifidobacteria rather than clostridia and *Bacteroides* spp. CS can be proposed as a new potential functional ingredient in consideration of the high content of soluble DF, the marked antioxidant activity, and the potential prebiotic activity.

KEYWORDS: Coffee, dietary fiber, silverskin, antioxidative activity, prebiotic property

INTRODUCTION

Dietary fiber (DF) is one of the main dietary factors contributing to consumers' well-being (*1*). Different claims on the healthy properties of DF have been released as epidemiological studies and have shown the importance of DF in different chronic diseases. To increase DF public consumption, new DF-enriched food has been developed in the past few years. The availability of DF from different sources, having noticeable physiological effects, promoted a number of investigations, particularly those aiming to use the DF present in food industry waste products (*2*).

The term DF indicates "the edible part of plants or analogous carbohydrates, which resist the hydrolysis by alimentary tract enzymes" (*1*). Fiber is partially bioavailable, being a portion of DF metabolized by intestinal microflora to volatile fatty acids in the hindgut (*3*). DF is divided into two categories according to its water solubility. Each category provides a different physiological effect (*1*). In fact, several studies have shown the role of insoluble fiber (lignin, cellulose, etc.) in the prevention of intestinal cancer (*4*), possibly because of improvement of the intestinal motility (*5*). In the past few years, many studies have demonstrated that soluble DF (pectin, inulin, oligofructose, etc.) is able to reduce glucose and sterol absorption from the intestine (*6*, *7*), to increase calcium absorption from the colon and rectum (*8*), to decrease serum cholesterol and postprandial blood glucose (*9*, *10*), and to support the growth of beneficial hindgut bacteria, thus exerting a prebiotic effect. As described by Gibson and Roberfroid (*3*), a prebiotic ingredient is not hydrolyzed by the human digestive enzymes in the upper gastrointestinal tract and it is able to beneficially affect the hindgut microflora by selectively stimulating the growth of some bacteria strains (mainly *Bifidobacterium* and *Lactobacillus*) that are recognized to promote host well-being.

So DF can be considered a nutritional factor that can contribute to consumers' health. The recommended intake of total DF is 25 g/day, of which about 25% should be soluble fiber (*11*). The average intake of DF in the Western countries is below this level, with the shortage of soluble DF more pronounced (*12*).

To increase the overall consumption of DF and to provide their appropriate balance, a new generation of functional food should be developed. The challenge is to create good products containing enough DF to satisfy local regulation for making a labeling and/or advertising claim and that elicits the desired physiological effects in the majority of the target population. Nowadays DF-rich ingredients mainly come from cereals because of their low cost and flexibility of use. Unfortunately, cereal fiber is mainly insoluble with the noticeable exception of oat products. However, other raw materials can be considered, particularly those, such as coffee, whose byproducts are low-cost and available worldwide.

Coffee is a major food commodity; therefore, coffee byproducts are amply available. The coffee silverskin (CS) is a tegument of coffee beans that constitutes a byproduct of the roasting procedure. CS is therefore obtained in coffee roasting plants located in the Western countries and presently used as combustible or fertilizer (*13*). Different studies have shown the healthy properties of coffee brews: the antioxidant capacity (*14*, *15*), the antibacterial and *ex vivo* protective activities (*16*, *17*), the anticarcinogenic effects (*18*), and the ability to increase plasma total glutathione (*19*). As CS is the outer layer of the

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roasted coffee beans, it is conceivable that some of the properties described for coffee brews are maintained also in CS.

The purpose of this work was to evaluate the potential use of the CS as a food ingredient by studying its chemical characteristics and investigating its antioxidant proprieties.

MATERIALS AND METHODS

Chemicals. Coffee silverskin samples have been obtained from different Italian roasting plants, and commercial samples of wheat bran and white wheat from Divella were used as control. *N*,*N*-Dimethyl-*p*-phenylenediamine (DMPD) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were from Fluka. An enzymatic kit to determine fiber amount was from Megazyme; it was constituted by an α-amylase (E-BLAAM) with an activity of 3000 Ceralpha units/mL, a protease (E-BSPRT) at a concentration of 50 mg/mL (350 tyrosine units/mL), and an amyloglucosidase (E-AMGDF) with an activity of 200 *p*-NP-*β*-maltoside units/mL. Bacteriological growth media were from Oxoid. Other chemicals if not specified were from Sigma−Aldrich. All solvents used were HPLC-grade from Merck.

Food samples (apple, peach, tomato) used for antioxidant activity comparison were prepared from fresh product and immediately freeze-dried. The antioxidant activity was determined on aqueous extracts obtained by dissolving in water at the same concentration (10 mg mL^{-1}) the different lyophilized materials.

Apparatus. Enzymatic incubation was performed in a water bath with shaking and cover (Stuart Scientific). A spectrometric apparatus UV 2100 (Shimadzu, Japan) was used to evaluate antioxidant capacity.

The amount of proteins was determined with a Kjeldahl apparatus (Falc Instruments) while fat quantity was determined in a Soxhlet apparatus. Melanoidins were determined after dialysis with a membrane with a cutoff of 12 000 Da (Prodotti Gianni).

Bacteriological growth media were autoclaved before their utilization. The fermenters were incubated in an anaerobic chamber (Don Whitley Scientific) under an atmosphere of N_2–CO_2–H_2 (80:10:10 v/v/v) at 37 °C and in an aerobic chamber at 37 °C.

Determination of Chemical Composition. Chemical composition of all samples was determined by official methods. In particular, the percentage of total sugars was determined after acid hydrolysis as reported in ref 20; reducing sugar, by the Fehling method; protein, by the Kjeldahl method; fat amount, with a Soxhlet apparatus; and moisture and dry weight, by gravimetric methods.

The amount of free phenols was determined after extraction with a mixture of 60:40 methanol/water by the Folin−Ciocalteus method, and they were identified by HPLC as described by Sacchi et al. (*21*). The amount of water-soluble melanoidins was determined by weighing the product obtained after dialysis with a membrane with a cutoff of 12 000 Da.

The amount of ochratoxin A was evaluated by an HPLC method as described by Solfrizzo et al. (*22*).

Determination of Total, Soluble, and Insoluble Fiber. The amount of soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) have been determined according to a gravimetric enzymatic method as previously described by Prosky et al. (*23*).

The procedure, performed in duplicate, consists of two main steps: enzymatic digestion and filtration. The first step is common for the determination of all type of fiber, while the washing steps during the filtration procedure vary with the kind of fiber investigated.

1. Enzymatic Digestion. Samples (1 g) were suspended in 40 mL of 0.05 mM Tris−Mes buffer, pH 8.2, and incubated at 100 °C for 35 min with 50 μL of α-amylase. After the solution cooled at 60 °C, 100 μL of the protease mixture was added to the solution, and this was incubated at 60 °C. Thirty minutes later, the reaction was stopped adding 5 mL of 0.56 M HCl with a pH value between 4.1 and 4.8. Finally 200 μL of amyloglucosidase was added and the solution was incubated at 60 °C for 30 min. To the hydrolyzed solution was added 300 mL of ethanol at 60 °C, and this solution was filtered after 1 h.

2. Filtration. A. Determination of TDF. TDF was obtained by filtering the ethanol solution through a crucible with a Celite filter. Subsequently, insoluble material was repeatedly washed as follows: three washings with 25 mL of ethanol (78%); two washings with 10 mL of ethanol (90%); and two washings with 10 mL of acetone.

Two identical aliquots were prepared. Both aliquots were placed at 103 °C overnight and then weighed. After, one of them was placed for 5 h in a muffle furnace at 525 °C and then weighed; the other one was used to quantify the protein amount by the Kjeldahl method. The protein amount was subtracted from TDF by application of the following formula:

$$\text{TDF (\%)} = \frac{[R - (p + A/100)R] - B}{100m}$$

All values are expressed in grams, where m = sample weight, R = total solids from m, p = protein weight, A = ashes weight from R, and B = weight of blank (enzymes + solvents without sample):

$$B = BR - BP - BA$$

where BR = total solids from B, BA = ashes from BR, and BP = protein from BR.

B. Determination of IDF. IDF was obtained as described for TDF, but an extensive washing with warm water was added. The enzymatically digested solution was filtered on the crucible and the insoluble material was washed as follows: once with 10 mL of distilled water at 60 °C; three times with 25 mL of ethanol (78%); twice with 10 mL of ethanol (90%); and twice with 10 mL of acetone.

The following steps were performed as previously described for TDF determination. The percentage of IDF was calculated from the same formula used for TDF evaluation.

C. Determination of SDF. SDF was obtained by adding 300 mL of ethanol at 60 °C to the filtered fractions collected from IDF separation. The SDF was precipitated and recovered after 1 h by filtration of the solution on the crucible. Pooled fractions were washed as follows: three times with 25 mL of ethanol at 78%; two times with 10 mL of ethanol at 90%; and two times with 10 mL of acetone. The following steps were performed as previously described for TDF determination. The percentage of SDF was calculated from the same formula used for TDF evaluation.

Determination of Antioxidant Activity. To measure antioxidant activity of each sample, aqueous and methanol extracts were obtained. The extractions were performed as follows: 1 g of each sample was added at 5 mL of distilled water or methanol and then put in a refrigerated centrifuge at 4000 rpm for 5 min. The procedure was performed twice. The supernatants were removed and after filtration (0.45 μm) were conserved at 4 °C.

Aqueous extracts were used for the DMPD assay, while methanol extracts were used for the ABTS assay. The water-soluble antioxidant activity was measured by the DMPD (*N*,*N*-dimethyl-*p*-phenylenediamine) method (*24*), while the lipophilic antioxidant activity was measured by the ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] method performed as previously described by Pellegrini et al. (*25*).

Determination of Prebiotic Properties. The ability of bacteria to utilize coffee silverskin (D) and glucose (control, E) was performed as described by Olano-Martin et al. (*26*) in static batch culture fermenters (in an anaerobic cabinet at 37 °C). Briefly, the procedure was as follows:

A. Bacteriological Growth Media. All agar substrates were prepared in triplicate following well-established procedures described by Olano-Martin et al. (*26*). In particular the substrates were nutrient agar (NA) for total aerobes, Wilkin's chalgren (WC) for total anaerobes, MacConkey agar 3 (MC) for coliforms, Rogosa agar (RG) for lactobacilli, reinforced clostridial agar (CL) for clostridia, Beeren's agar (BR) for bifidobacteria, and Brucella agar (BC) for *Bacteroides* spp.

B. Inoculum. Three different healthy donors, who had not taken antibiotics for 3 months beforehand, provided fresh fecal material. A 5% (w/v) dilution of fecal sample in sterile, prereduced phosphate-buffered saline (PBS) was used as inoculum for the batch culture. The final concentration of fecal slurry in the batch cultures was 1% (w/v).

C. Microbiological Culture Techniques. Samples (0.5 g) were dissolved in 5 mL of PBS at pH 7.0 and then added at 44 mL of chemostat basal medium (prepared as described in ref *27*) and 1 mL

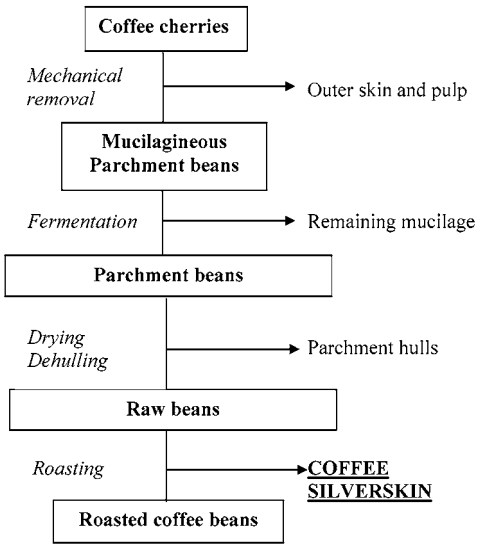

Figure 1. Sketch of the production of coffee silverskin (CS) from wet processing.

of fecal slurries. Fresh feces were always used to prepare the inoculum for the batch cultures. Aliquots (1 mL) of each batch culture were removed after 0, 5, 10, and 24 h and serially diluted from 10^{-1} to 10^{-9} for bacteriological analysis. After mixing, 20 μL was plated onto a quarter of an agar plate, four dilutions per plate, onto triplicate plates. Plates were then incubated at 37 °C for 24−72 h aerobically or 72−120 h anaerobically. Bacterial colonies were differentially counted, according to morphology, with a colony counter and summed, and the total log counts per milliliter of sample was calculated. Colonies were tentatively identified by morphotype and selected microscopic/biochemical traits as described by Gibson and Roberfroid (*3*). All procedures were carried out in an anaerobic cabinet (10% H_2/10% CO_2/80% N_2).

D. Colony Count. The data obtained (number of colonies and dilution value) were processed by application of the formula log (count × 50) × 10^{dil}, where count is the number of colonies, 50 is the volume (in milliliters) of nutrient medium + sample, and dil is the dilution of the count.

Statistical Analysis. Differences between bacterial counts at each fractional time fermentation for each substrate and antioxidant activity values were tested for significance by the Turkey test, which allowed a multiple comparison among the data to individualize the significant differences among the same. Differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Coffee silverskin (CS) is a tegument that covers each coffee bean, and it is obtained both by dry and wet coffee bean postharvest procedures. In Figure 1 the layout of the wet process is shown (*13*). CS is the only coffee byproduct obtained in the Western countries as a waste material of the roasting plants.

In Table 1 the proximal data of CS composition, compared to those of cereal byproducts and wheat wholemeal, are shown. These data refer to CS samples obtained from three different 100% *Arabica* beans roasted in three different plants. Data are the mean of the determination performed on the different CS. Standard deviation is around 5% for the different parameters, thus suggesting that, among *Arabica* varieties, the composition of CS is comparable.

CS has a marked amount of protein, fats are very low, and reducing carbohydrates are almost absent. The ash value is high, suggesting an important mineral content. Polysaccharides represent the main components of CS: it is very likely that, as described by Nunez and Coimbra (*27*, *28*) for coffee beans, they

Table 1. Chemical Composition and Fiber Amount of Coffee Silverskin in Comparison with Those of Other Fiber-Rich Materials[a]

parameters	coffee silverskin	wheat bran	oat bran[b]	wheat wholemeal
proteins	18.6 ± 0.6	14.1	22.2	11.9
fats	2.2 ± 0.1	5.0	11.4	1.9
carbohydrates	62.1 ± 1.6	70.4	27.2	78.0
reducing sugar	0.21 ± 0.01	26.8	4.2	68.4
moisture	7.30 ± 0.4	8.0	7.8	13.4
ashes	7.0 ± 0.2	2.5	4.1	0.7
ochratoxin A	<4 ppb[c]	nt	nt	nt
TDF	62.4 ± 0.6	43.5 ± 0.1	21.2	9.6
IDF	53.7 ± 0.2	41.8 ± 0.0	12.7	9.1
SDF	8.8 ± 0.4	1.7 ± 0.5	8.5	0.5

[a] Values are given per 100 g of product. [b] Data from ref *35*. [c] Maximum level suggested for Italy by the Istituto Superiore Sanità.

are mainly arabinogalactans and galactomannans. Since CS a polysaccharide-rich material, it is very important to evaluate the ratio between soluble and unsoluble DF to evaluate its use as potential functional ingredient. The amount of fiber contained in coffee silverskin was determined as described by Prosky et al. (*23*) and results are also shown in **Table 1**. Upon comparison of CS data with those of other cereal-based ingredients, it is evident that CS has the highest amount of TDF (62.2%). The amount of SDF of CS is 8.7%; this means that SDF represents about 14% of the total fiber. The amount of SDF is comparable with that of oat bran, but the latter has only 21% TDF. The compositional data of CS suggest its possible use as a functional ingredient to develop fiber-rich foods containing a substantial amount of SDF. In fact, the intake of SDF should increase in Western countries to meet nutritional requirements (*12*).

The concentration of phenol compounds present in the CS has been also determined. Phenolic compounds were extracted in 60:40 methanol/water, quantified by Folin−Ciocalteus, and identified by HPLC (*21*). A total amount of 1.1 mg of phenol compounds (expressed as chlorogenic acid)/100 g of product was detected, and HPLC data indicate that chlorogenic and caffeic acid are the main free phenols present in the CS. The concentration of free phenolic compounds is much lower than that detectable in roasted beans. This is likely due to the higher temperature experienced by the outer layer of the beans during roasting, which causes fast degradation of phenol compounds. Interestingly, when the detection of phenol compounds was performed after NaOH hydrolytic treatment, no increase of the phenol concentration was detectable. It should be outlined that when the same treatment is performed on cereal fiber, phenol compounds, mainly ferulic acid, are released as they are linked to the carbohydrate fiber through ester bonds (*29*). This figure suggests that phenolic compounds present in the CS are either free or incorporated into the polymer backbone, as previously found in coffee brews (*14*).

The amount of water-soluble melanoidins in CS is about 4.5%. This concentration is comparable to that observed in coffee brews. In fact, the standard yield of hot water extraction preparing coffee brews is about 17%, and melanoidins represent about 25% of the dry weight of the brews.

Antioxidant Capacity. The antioxidative activity of aqueous and methanol extracts from coffee silverskin were evaluated by two methods based on the ability to scavenge two radical cations, $ABTS^{\bullet+}$ and $DMPD^{\bullet+}$. These two methods were particularly effective to measure antioxidant properties of compounds contained in CS and cereals. The ABTS assay allows investigation of the antioxidant capacity of components extracted

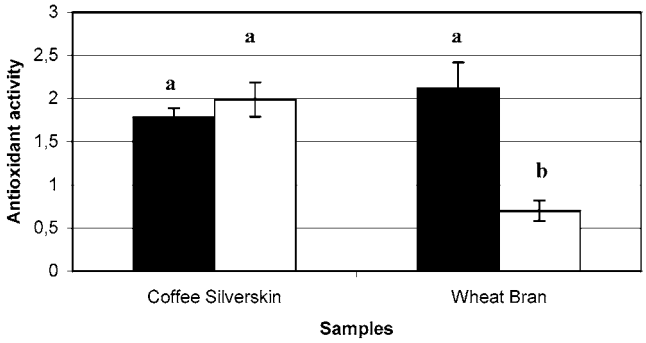

Figure 2. Antioxidant activity of CS and wheat bran expressed as millimoles of Trolox/100 g of product (ABTS$^{\bullet+}$, solid bars) and millimoles of ascorbic acid/100 g of product (DMPD$^{\bullet+}$, open bars).

Table 2. Comparison of Antioxidant Activity of CS with That of Coffee Brew and of Other Fresh Vegetables (ABTS$^{\bullet+}$ Assay)

vegetables	mmol of Trolox/100 g of dry weight
tomatoes	2.16
peaches	2.15
apples	2.78
coffee brews	1.63
coffee silverskin	1.92

in methanol, while the DMPD$^{\bullet+}$ method is applied to evaluate the activity of water-soluble compounds.

As shown in **Figure 2**, CS methanol extracts have an antioxidant activity similar to that of wheat bran, which is known to have a very high antioxidant activity (*30*). Moreover, CS showed a value of antioxidant activity higher than that of wheat bran when the aqueous extract was assayed. This difference is likely related to the better water solubility of CS antioxidants.

In **Table 2** the comparison among antioxidant activity, expressed as millimoles of Trolox per 100 g of dry weight, of coffee silverskin, coffee beverage, and several fresh vegetables prepared as described above is shown. CS has an antioxidant activity comparable to that of fresh vegetables, which are well-known antioxidant sources.

Antioxidant activity of coffee silverskin may be explained by considering that this tegument keeps part of the polyphenolic compounds that are normal constituents of coffee beans and, as suggested by several studies (*14*, *15*, *31*), by the presence of compounds, mainly melanoidins, that are formed through Maillard reaction during roasting. The high antioxidant activity measured for CS suggests that in this food matrix antioxidant compounds are covalently bound to the carbohydrate backbone, forming a fiber−antioxidant complex. For example, in cereals, this fiber−antioxidant complex contains many ferulic acid esters (*29*, *32*). In CS the phenolic compounds, mainly chlorogenic acid, which are present in a huge amount in green coffee beans, react during roasting with the polysaccharide components, forming a mixture of dark polymers named melanoidins (*33*). These melanoidins are rich in phenolic groups, which are still able to exert antioxidant activity (*14*). Therefore, it is possible that, at least in part, the beneficial effects usually attributed to fiber are actually due also to the antioxidant compounds linked to the carbohydrate structure. Not only for CS but also for many cereal fibers, increasing evidence suggests that attention should be focused on the antioxidant−fiber complex more than on the fiber moiety only.

Prebiotic Efficiency. Evaluation of the prebiotic activity of CS was obtained by an in vitro assay aimed to evaluate the effects of CS on hindgut microflora. The experiments were performed in an anaerobic cabinet to create the same atmosphere presents in the intestine, and the lumen was represented by serum bottles containing nutrient medium and fecal bacteria to create conditions similar to that of the human colon.

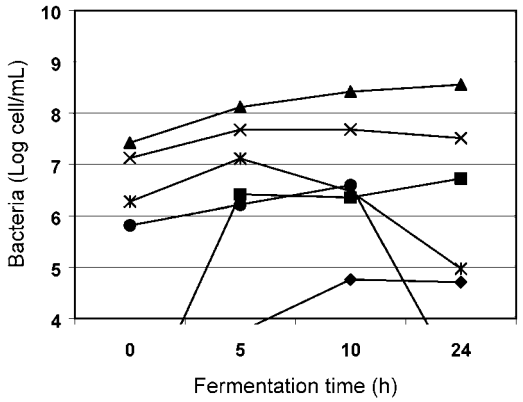

Figure 3. Bacterial growth during 24 h of fermentation in the presence of CS as unique carbon source: (▲) Total anaerobes, (×) bifidobacteria; (*) *Bacteroides* spp.; (●) clostridia; (■) coliforms; (◆) total aerobes. The number of lactobacilli cells is below the value of four, so their growth is not visible on the graph.

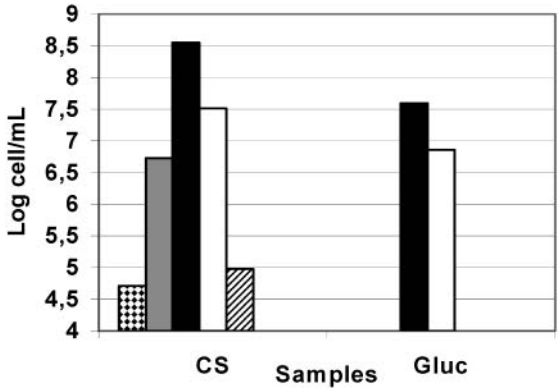

Figure 4. Comparison between the bacterial growth in the presence of CS and of glucose as unique carbon source (control). Dotted bar, total aerobes; gray bar, coliforms; black bars, total anaerobes; white bars, bifidobacteria; striped bar, *Bacteroides* spp.

The procedure described in the Experimental Section allows evaluation of the growth of different groups of bacteria, which were used as a marker of gut functionality: bifidobacteria, lactobacilli, clostridia, coliforms, and *Bacteroides* spp. The first two groups promote healthy human conditions while clostridia, coliforms, and *Bacteroides* spp. may be seen as nondesirable presences in gut microflora (*3*, *34*). In **Figure 3** are shown the results obtained from the static batch culture, where CS was used as carbon source. An increase in the number of microorganisms was observed during fermentation. In particular the increase of the numbers of total anaerobic and aerobic bacteria demonstrate the ability of microorganisms to use the coffee silverskin as a source of nitrogen and carbon. By comparison of the bacterial counts on selective growth media, it is possible to observe a predominant development of bifidobacteria with respect to the other microorganisms. In fact, coliforms showed a limited growth, while *Bacteroides* spp. and clostridia growth was inhibited. On the other hand, *Lactobacillus* spp. showed a limited aptitude to use CS for their growth, therefore their cell number per milliliter is below 10^4 and they are not shown in **Figure 3**.

Bifidobacteria growing on silverskin were identified by Gram stain and by evaluating morphological properties under the

microscope (100× magnification). Gram coloration confirmed that the microorganisms were Gram-positive, while electronic microscope showed the presence of pleomorphic rods typical of bifidobacteria strains (data not shown).

Figure 4 shows a comparison of the growth of the different bacterial species on coffee silverskin (CS) and on glucose after 24 h of fermentation. Glucose is used as a positive reference as it is known that in this condition it promotes the growth of bifidobacteria.

The graph shows the predominance of bifidobacteria among the several anaerobic species. By comparison of the results, it is possible to observe that the number of total anaerobic bacteria and bifidobacteria is quite similar in both tested samples, although the CS sample also allowed the growth of coliforms.

CONCLUSION

Data shown in this work concurrently suggest that CS has the potential to be considered a functional ingredient for the development of fiber-rich functional food:

(i) It has a low amount of fats and reducing carbohydrates;

(ii) it has a high amount of TDF with an absolutely relevant amount of SDF; and

(iii) it has a marked antioxidant activity due to the peculiar composition of its fiber, which is rich in Maillard reaction products and maintains some phenolic compounds.

Moreover, preliminary experiments also shown that CS preferentially supports the growth of bifidobacteria in vitro, thus suggesting that the consumption of CS may have some prebiotic effects.

ABBREVIATIONS USED

CS, coffee silverskin; DF, dietary fiber; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); PBS, phosphate-buffered saline.

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