International Food Research Journal 24(5): 2246-2252 (October 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluation of antioxidant, anti-pathogenic and probiotic growth stimulatory activities of spent coffee ground polyphenol extracts

Klangpetch, W.

Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, 65000, Thailand

Article history

Received: 26 August 2016
Received in revised form: 29 September 2016
Accepted: 30 September 2016

Keywords

Coffee ground
Polyphenol extract
Antioxidant
Antimicrobial
Probiotic

Abstract

Antioxidant, anti-pathogenic and prebiotic activities of polyphenol extracts from spent coffee grounds (SCGE) *(Coffea arabica)* obtained after drip-brewing were investigated. Extraction experiments were done by a conventional solid–liquid method, using ethanol as a solvent at a different concentration ranges from 0 to 100%. Extraction of SCGE using 60% ethanol exhibited the highest total phenolic content of 146.78 mg gallic acid equivalents/g SCGE and antioxidant activity of IC_{50} 2.83 mg/ml. The minimum inhibitory concentration of SCGE was demonstrated against *Escherichia coli, Salmonella* Typhimurium, *Bacillus cereus* and *Staphylococcus aureus* at 12.5 mg/ml. In comparison, SCGE did not express an inhibitory effect on the common probiotic bacteria, *Lactobacillus* spp. and *Bifidobacteria* spp. As such, spent coffee grounds can be recycled and used as a source of alternative natural functional food ingredients, while also providing sustainable management of an industrial by-product.

Introduction

Interest in utilizing agro-food by-products as a potential source of natural antioxidants and functional food ingredients has been increasing in recent years (China *et al*., 2012; Montella *et al.*, 2013). Spent coffee grounds (SCG) are an abundant by-product from the coffee brewing industry. Their chemical characterization is based on fibers that exist in coffee bean cell walls such as cellulose and hemicelluloses, fat, proteins, polyphenols and minerals. Moreover, during the roasting process, there are different bioactive products formed from a Maillard reaction, such as melanoidins (Borrelli *et al.*, 2004). These organic substances make SCG highly pollutant due to the great demand of oxygen used to be degraded (Kondamudi *et al.*, 2008). Moreover, caffeine, polyphenols or tannins exhibit a toxic effect from these by-products, especially as a soil pollution hazard when they are released into the land (Ricardo *et al*., 2013). Therefore, it is worthwhile to pursue methods to improve utilization of these currently harmful wastes to achieve a zero-waste strategy.

Previous studies found considerable evidence of health benefits for healthy adults as a result of drinking moderate amounts of coffee, partially attributed to the antioxidant activity of polyphenols (Vignoli *et al*., 2011). Some of these activities exist even in SCG (Jiménez-Zamora *et al*., 2015). SCG can be used for pellet production due to its high calorific power of around 5000 kcal/kg (Silva *et al*., 1998). Other valuable alternative uses include in energy production such as biodiesel, methanol and hydrogen production. SCG can also be used as a substrate for the fermentation process (Ramalakshmi *et al*., 2009).

Along with the significant phytochemicals found in a coffee brew, a coffee beverage can also be a good source of dietary fiber. Díaz-Rubio and Saura-Calixto (2010) found that compared with other beverages, coffee contained significantly greater amounts of soluble dietary fiber of 0.47– 0.75 g/100 ml, coupled with antioxidant phenolic compounds. Interestingly, these phenolic compounds are also known for their antibacterial activities. Furthermore, coffee consumption seems to increase the population of *Bifidobacteria* and their metabolic activity, indicating that coffee might have some prebiotic effects (Jaquet *et al*., 2009).

Although there are some studies showing the antioxidant and antibacterial activities of coffee and SCG, there is a lack of information regarding the most effective conditions for extraction of SCG and reports that consider probiotic growth promotion by SCG for *Lactobacilli* and *Bifidobacteria*. This study aimed to evaluate the effect of ethanol concentration in solvent extraction of SCG on total phenolic content, antioxidant anti-pathogenic and probiotic growth promotion using several species of *Lactobacilli* and

*Corresponding author.
Email: *wannapornk@nu.ac.th*
Tel/Fax: +66 55 96 2742

Bifibobacteria.

Materials and Methods

Chemicals and reagents

Ethanol and dimethyl sulfoxide (DMSO) were obtained from Labscan, Poland. Folin-Ciocalteu reagent was purchased from Loba Chemie, India. Gallic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Chemicals, Germany. Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA) and MRS Broth were purchased from Himedia Laboratories, India.

Bacterial strains and culture conditions

The pathogenic bacterial strains used in this study were *Bacillus cereus* TISTR 687, *Staphylococcus aureus* TISTR 118, *Escherichia coli* TISTR 780, and *Salmonella* Typhimurium TISTR 292 purchased from Microbiological Resources Center of the Thailand Institute of Scientific and Technological Research. The indicator bacteria were cultured on TSA and subcultured in TSB at 35°C for 24 h under aerobic condition before use. Each bacterial suspension was then adjusted to obtain a final concentration of 10^7 CFU/ml. The probiotic bacterial strains used in this study were *Lactobacilli*; *Lactobacillus bugaricus* TISTR 892, *Lactobacillus casei* TISTR 1463, *Lactobacillus plantarum* TISTR 541, *Lactobacillus acidophilus* TISTR 1034, *Lactobacillus brevis* TISTR 855 and *Bifidobacteria; Bifidobacterium bifidum* TISTR 2129 and *Bifidobacterium breve* TISTR 2130. They were cultured on MRS agar media and then transferred to MRS broth. 0.05% L-cysteine for *Bifidobacteria* was also added to the MRS. All *Bifidobacteria* strains were grown anaerobically at 35°C for 48 h in a 5% CO_2 incubator (Shel Lab 2323, Sheldon Manufacturing, Cornelius, OR).

Preparation of spent coffee ground polyphenol extracts (SCGE)

SCG *(Coffea arabica)*, the residue of coffee drip-brewing was obtained from a coffee shop in the Phitsanulok province of Thailand. The SCG was dried in a hot air oven at 60°C until the moisture content reached ≤ 5% and was stored at -20°C until used.

SCG was extracted by soaking with ethanol at concentrations of 0, 20, 40, 60, 80 and 100% in a ratio of 1:30 with shaking at 60°C for 90 mins. The extracts were then filtered through Whatman No. 4. filter paper (Whatman, Kent, UK). The filtrates were evaporated under a rotary-vacuum evaporator at 50°C to dryness (Buchi RTE-221, Flawil, Switzerland) and freeze-dried (Labconco Co., USA) to obtain the SCGE. The SCGE was then weighed to determine dry basis extraction yields.

Determination of total phenolic content (TPC)

Total phenolic content (TPC) of the SCGE was measured according to the Folin–Ciocalteu method of Singleton and Rossi (1965) with a slight modification. Briefly, the extract was dissolved in methanol to a concentration of 5 mg/ml, then the 100 µL of the mixture was mixed with a Folin–Ciocalteu reagent (1:9 of Folin–Ciocalteu reagent:distilled water). After that, the mixture was mixed with 1.0 ml of sodium carbonate solution 15% (w/v) and vigorously vortexed for 2 minutes. Methanol was used to make up the final volume to 5.0 ml and left in darkness at room temperature for 2 hours. After that the absorbance of the mixture was measured and recorded at 750 nm using a spectrophotometer (Thermo Scientific, Genesys, USA). The calibration curve was made using Gallic acid as a standard. The amount of total phenolic was calculated and reported as mg gallic acid equivalents (GAE) per gram of sample.

Determination of DPPH radical scavenging activity

SCGE free radical scavenging activity was determined by a DPPH radical scavenging assay, according to the modified method of Brand-Williams *et al*. (1995). Briefly, 100 µM of DPPH in methanol was prepared and 2 ml of this solution was added to 1 ml of 5 mg/ml of SCGE sample solution. The reaction mixture was shaken well and left in darkness for 30 min at room temperature. The resulting solution was then measured for absorbance and recorded at 517 nm using a spectrophotometer. Each sample was measured in triplicate and calculated for average values. The percentage of radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of samples after reaction. The free radical scavenging activities of the extracts were expressed as an Inhibition Concentration 50 value (IC_{50}). The IC_{50} value was defined as concentration in mg/ml of SCGE sample that inhibits 50% of DPPH radical formation.

Assay for antibacterial activity

Antibacterial activity for SCGE was determined using an agar disc diffusion method (Oke *et al.*, 2009). A bacterial suspension of 100 µL was spread

Table 1. Effects of ethanol concentration on the extraction yield (%), TPC (mg GAE/g) and IC_{50} (mg/ml) of SCGE

Ethanol (%)	Extraction Yield (%)	TPC (mg GAE/g)	IC_{50} (mg/ml)
0	4.28±0.32^{c}	77.50±1.00^{c}	9.48±0.29^{a}
20	6.24±0.52^{a}	118.78±1.22^{b}	5.45±0.13^{b}
40	5.43±0.02^{b}	123.28±0.89^{b}	6.58±0.23^{b}
60	5.54±0.55^{b}	146.78±0.98^{a}	2.83±0.03^{c}
80	7.3±0.21^{a}	96.45±1.33^{b}	3.42±0.02^{c}
100	5.25±0.13^{b}	99.70±2.14^{b}	3.31±0.01^{c}

Means with different letters are significantly different ($p < 0.05$).

homogeneously onto the surface of TSA. Sterile filter paper discs 6 mm diameter size were placed on the agar surface and dropped with 6 µL of the 50 mg/mL of the SCGE in DMSO (the DMSO was checked for no inhibition effect against the tested bacteria). After leaving at room temperature for 15 min to allow for diffusion time, the plates were incubated at 35°C for 24 h. The presence of an inhibition zone was observed.

A micro broth dilution technique was performed to determine the minimum inhibitory concentration (MIC) of each SCGE (Jorgensen and Turnidge, 2007). The extract was added in a two-fold dilution manner in a 96-well plate, ranging from 0.2 to 50 mg/ml in 50 µL of each bacterial suspension. After incubation at 35°C for 24 h, OD_{630} was measured by a microplate reader (SM600, Drawell International Technology Co., Ltd., China) and the lowest concentration of the SCGE required to suppress the visible growth of the target pathogenic bacteria was designated as the MIC.

Effect of SCGE on the growth of probiotic bacteria

The effect of SCGE on the growth of seven strains of probiotic bacteria was evaluated by the plate count method according to Das *et al*. (2011). Briefly, the experimental and control cultures were diluted to the appropriate concentrations in MRS broth and were plated on MRS agar medium and cultured in a 5% CO_2 incubator at 35°C for 48 h. The effect of SCGE at 0.5 and 1% (w/v) on the growth of probiotic bacteria was determined by comparing the viable cell counts (CFU/ml) of the sample in the modified MRS adding the SCGE as a unique carbon source instead of dextrose against those obtained from a commercial MRS. The viable cell counts at the initial stage (0 hr) of the culture were also determined.

Statistical analysis

All experiments were performed in triplicate. The average values with standard errors were calculated and reported. Analysis of variance (ANOVA) was used to compare mean values using SPSS software (version 13.0). Means and standard errors were calculated. Significance was defined at $p < 0.05$.

Results and Discussion

Effect of ethanol concentration on extraction yield, TPC and DPPH radical scavenging activity

Phenolic compounds, which are secondary metabolites produced by most plants, are generally responsible for the antioxidant activity of many fruits and vegetables. The Folin–Ciocalteu procedure has been proposed to rapidly estimate the level of TPC in foods and supplements (Prior and Schaich, 2005). The effects of ethanol concentration on percent yield, TPC (mg GAE/g) and IC_{50} (mg/ml) of SCGE are shown in Table 1. For comparison, an assay using only distilled water as an extraction solvent was also performed. However, the use of ethanol as a solvent gave better extraction results than the use of only water. Phenolic compounds are more soluble in an organic solvent that is less polar than water.

As the concentration of ethanol was increased from 0 to 60%, TPC content increased. However, TPC dropped for 80% and 100% ethanolic extracts. The highest TPC recovered by ethanol extraction corresponded to a 60% ethanolic extract (146.78 ± 0.98 mg GAE/g) with a significant difference compared to other samples. The same trend was expressed for $IC_{50,}$ the antioxidant marker, showing the lowest for 60%, indicating the highest antioxidant capacity (2.83 ± 0.03). In contrast, the difference for %yield in each sample seemed unrelated to ethanol concentration.

Affiliated with dietary fiber and proteins, different health-related compositions such as phenolic compounds, have been reported to be present in SCG (Cruz *et al.*, 2011; Bravo *et al*., 2013). SCGE antioxidative properties could be associated with the presence of caffeine, trigonelline and chlorogenic

Table 2. Inhibition effects of SCG extracted with ethanol at different concentrations against pathogenic bacteria

Indicator bacteria	Inhibition zone diameter (mm)/Sample 100 mg/mL					
	0%	20%	40%	60%	80%	100%
B. cereus	-	-	-	9.8±0.0	7.4±0.3	7.2±0.1
S. aureus	-	-	-	9.4±0.2	8.0±0.2	6.9±0.0
S. Typhimurium	-	-	-	8.9±0.0	-	6.9±0.2
E. coli	-	-	-	9.1±0.1	-	7.0±0.1

Table 3. MIC of CF and CG extracted with ethanol at different concentrations against pathogenic bacteria.

Indicator bacteria	MIC (mg/mL)					
	0%	20%	40%	60%	80%	100%
B. cereus	-	-	-	12.5	50	50
S. aureus	-	-	-	12.5	12.5	50
S. Typhimurium	-	-	-	12.5	-	100
E. coli	-	-	-	12.5	-	100

acids (Franca *et al.*, 2011). Chlorogenic acids, a type of Caffeoylquinic acid, are the main components of phenolic compounds whose consumption may provide remarkable health benefits, including reduced incidence of diabetes or atherosclerosis (Natella *et al.*, 2002; Narita and Inouye, 2011).

Antimicrobial activity

SCGE antibacterial activity was measured using a disc diffusion assay followed by a micro broth dilution assay in order to determine MIC. Firstly, the susceptibility of each indicator pathogenic bacteria to the SCGE was evaluated by observing the inhibition zone (Table 2). The results showed that a lower concentration of ethanol showed less inhibition. SCGE from 60 to 100% showed an inhibition effect against *B. cereus* and *S. aureus*, while 60 and 100% showed inhibition against *S.* Typhimurium and *E. coli*. Various degrees of inhibition against pathogenic bacterial strains were reported as MIC and the results are given in Table 3.

The lowest MIC was for 60% SCGE at 12.5 mg/ml against all of the indicator strains. This result gave 100 times higher activity toward *Sesamum indicum* honey polyphenol extract (Das *et al*., 2015). Based on ethanol extraction, a 60% ethanolic extract exhibited the highest antioxidant along with antimicrobial activities. Hence, there may be a correlation in these two parameters, and between antioxidant and antimicrobial activities. Some studies have also investigated phenolic compounds composed from various plant origins, which may inhibit several food-borne pathogenic microorganisms, and TPC was highly correlated with antimicrobial ability (Radha *et al.*, 2014). Multiple modes of action may be involved in the antimicrobial activities of phenolic compounds. Phenolic compounds can degrade the cell wall of bacteria, destroy the cytoplasmic membrane, cause leakage of cellular components in cytoplasm, change fatty acid and phospholipid constituents, affect the synthesis of DNA and RNA and disrupt protein translocation (Shan *et al*., 2007).

The roasted coffee extract exhibited antibacterial activity against several microorganisms, such as Streptococcus mutans (Daglia *et al*., 2007) and several strains of enterobacteria. This is probably due to the antibacterial activity of several coffee characteristic components, such as chlorogenic acid, caffeine, trigonelline, protocatechuic acid and caffeic acid (Almeida *et al.*, 2006), as well as melanoidins generated during the coffee bean roasting process (Bravo *et al.,* 2012). Coffee melanoidins are known to behave as a bacteriostatic as well as bactericide compound based on their concentration and at the concentration present in SCGE. Coffee melanoidins have been proven to have the ability to exert some kind of antimicrobial activity (Rufián-Henares and Cueva 2009). The main mechanism for the antimicrobial activity of melanoidins is related to their chelating activity, which affects bacterial cell walls.

In contrast to previous findings reporting that assays of SCGE extracted with hot water showed that SCG was only active against *B. cereus*, but not *E. coli,* SCGE extracted with ethanol in this study showed inhibition against *E. coli* and *S.* Typhimurium, indicating activity against both Gram positives; *B. cereus* and *S. aureus* and Gram negatives; *E. coli* and *S.* Typhimurium. Therefore, the SCGE in this

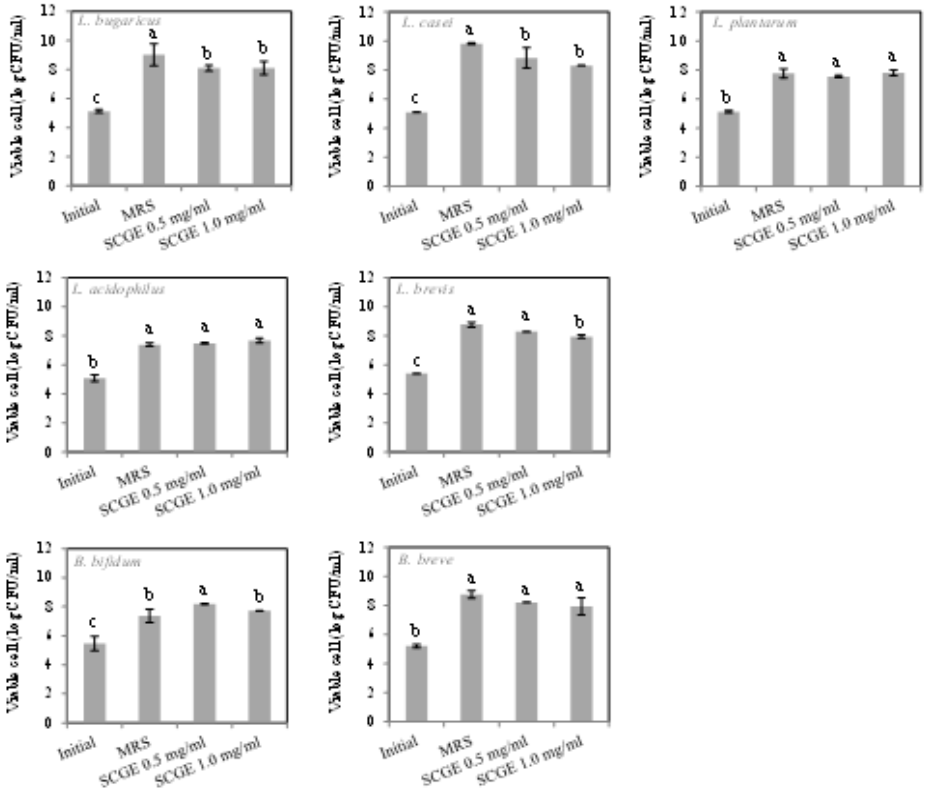

Figure 1. Growth stimulatory effect of SCGE on probiotic bacteria.

study may provide higher permeability that conquers the outer membrane barrier of Gram negatives, which made them less susceptible to antimicrobial substances compared to Gram positives (Nikaido, 1994).

Growth stimulatory effect on probiotic strains

This study determined the quantitative probiotic growth promoting activity of SCGE at a concentration of 0.5 and 1.0% (w/v) by using a plate count method. SCGE at both concentrations was added for preparation of modified MRS by substituting the dextrose, the carbon source present in MRS broth. The results from Figure 1 showed that all probiotic strains were growth-induced by SCGE at a similar level to the control (probiotic strains grown only in MRS broth) for both *Lactobacilli* and *Bifidobacteria.* For *L. plantarum, L. acidophilus, L. brevis* and *B. breve*, the bacteria grown in the media supplemented with SCGE as an alternative carbon source showed no significant differences in viable cell count when compared with commercial MRS media. Interestingly, *B. bifidum* showed log 8.19 ± 0.02 CFU/ml, indicating further growth stimulation in 0.5 mg/ml SCGE-supplemented media than that of MRS media (log 7.37 ± 0.45 CFU/ml). Moreover, the concentration of 0.5 mg/ml of SCGE gave a more notable stimulating effect in some probiotic strains such as *L. brevis* and *B. bifidum*.

Roasted coffee was composed on a dry basis by carbohydrates at 38–42%, melanoidins at 23%, lipids at 11–17%, protein at 10%, minerals at 4.5–4.7%, chlorogenic acid at 2.7–3.1%, aliphatic acids at 2.4–2.5%, caffeine at 1.3–2.4%, and other components (Jiménez-Zamora *et al*., 2015). Due to the complexity of its molecules, melanoidins have unknown structure showing high antioxidant activity with high molecular weight compounds. They are generated through a Maillard reaction from the combination of sugars and amino acids or caramelization of carbohydrates. It was previously stated that although some of the melanoidin was extracted during coffee brewing, the majority of the melanoidins, approximately 75% were still existed in SCGE and could be responsible for some of the biological activities of the SCGE. Furthermore, among carbohydrates, the major macronutrients found in SCGE, dietary fiber was the most critical fraction comprising 62%, with insoluble fiber predominant (Jiménez-Zamora *et al*., 2015). Coffee beverages contain significant amounts of soluble fiber, mainly discovered as galactomannans and arabinogalactan, proteins and phenolic compounds, and chlorogenic acids that are well utilized by human faecal microbiota (Umemura *et al*., 2004; Gniechwitz *et al*., 2007). Although regular coffee drinking has been regularly considered as containing low nutritional value, it has also been shown to have

high impact on several aspects of health. Probiotic bacteria, especially *Bifidobacteria* is known for their low growth ability when presenting in milk due to their lack of proteolytic activity. As such, they require assistance from essential growth factors such as peptides and amino acids to enhance their growth (Klaver *et al*., 1993). Marhamatizadeh *et al.* (2014) reported that 0.4% of the ethanolic extract of coffee enabled *B. bifidum* grows well in milk and yoghurt without further nutrient supplementation. Therefore, this finding will be useful in incorporation of SCGE in milk and other products in order to promote of the growth of Bifidobacteria.

The finding was relevant to a previous study showing that *Sesbania grandiflora* flower polyphenol extract induced a significant increase of *L. acidophilus* grown in culture media (China *et al*., 2012). The results showed that starting from 0.01% (w/v), both soluble and insoluble dietary fiber in hazelnut skin significantly improved the growth of *Lactobacillus plantarum* and *Lactobacillus crispatus* during fermentation toward control (Montella *et al*., 2013).

Conclusion

This study showed that a coffee brewing by-product, spent coffee grounds, has high total phenolic content that provides benefit to human health through antioxidant and anti-pathogenic activities, especially when using 60% ethanol as the extract solvent. Furthermore, the extract potentially exhibited prebiotic activity in terms of inducing probiotic growth at low concentrations. Therefore, this matrix has potential as a source of an alternative functional ingredient for both foods and nutraceutical products containing probiotic organisms.

Acknowledgement

The financial supported for this study was kindly provided from Naresuan University.

References

Almeida, A. A. P., Farah, A., Silva, D. A. M., Nunan, E. A. and Glória, M. B. A. 2006. Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. Journal of Agricultural and Food Chemistry 54: 8738–8743.

Borrelli, R. C., Esposito, F., Napolitano, A., Ritieni, A. and Fogliano, V. 2004. Characterization of a new potential functional ingredient: coffee silverskin. Journal of Agricultural and Food Chemistry 52: 1338–1343.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28: 25–30.

Bravo, J., Juaniz, I., Monente, C., Caermmerer, B., Krow, L.W. and De Pe~na, M.P. 2012. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. Journal of Agricultural and Food Chemistry 60: 12565–12573.

Bravo, J., Monente, C., Juaniz, I., De Pe~na, M. P. and Cid, C. 2013. Influence of extraction process on antioxidant capacity of spent coffee. Food Research International 50: 610–616.

China, R., Mukherjee, S., Sen, S., Bose, S., Datta, S. and Koley, H.

2012. Antimicrobial activity of Sesbania grandiflora flower polyphenol extracts on some pathogenic bacteria and growth stimulatory effect on the probiotic organism *Lactobacillus acidophilus*. Microbiological Research 167: 500–506.

Cruz, R., Cardoso, M. M., Fernandes, L., Oliveira, M., Mendes, E. and Baptista, P. 2012. Espresso coffee residues: a valuable source of unextracted compounds. Journal of Agricultural and Food Chemistry 60: 7777–7784.

Daglia, M., Papetti, A., Grisoli, P., Aceti, C., Spini, V. and Dacarro, C. 2007. Isolation, identification, and quantification of roasted coffee antibacterial compounds. Journal of Agricultural and Food Chemistry 55: 10208–10213.

Das, A., Data, S., Mukherjee, S., Bose, S., Ghosh, S. and Dhar, P. 2015. Evaluation of antioxidative, antibacterial and probiotic growth stimulatory activities of *Sesamum indicum* honey containing phenolic compounds and lignans. Food Science and Technology 61: 244–250.

Díaz-Rubio, M. E. and Saura-Calixto, F. 2007. Dietary fiber in brewed coffee. Journal of Agricultural and Food Chemistry 7: 1999-2003.

Franca, A. S., Oliveira, L. S., Mendonça, J. C. F. and Silva, X. A. 2005. Physical and chemical attributes of defective crude and roasted coffee beans. Food Chemistry 90: 89–94.

Gniechwitz, D., Reichardt, N., Blaut, M., Steinhart, H. and Bunzel, M. 2007. Dietary fiber from Coffee beverage: degradation by human fecal microbiota. Journal of Agricultural and Food Chemistry 55: 6989–6996.

Jaquet, M., Rochat, I., Moulin, J., Cavin, C. and Bibiloni, R. 2009. Impact of coffee consumption on the gut microbiota: a human volunteer study. International Journal of Food Microbiology 130: 117–21.

Jiménez-Zamora, A., Silvia, P. and Rufián-Henares, J. A. 2015. Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. LWT Food Science and Technology 61: 12–18.

Jorgensen, J. H. and Turnidge, J. D. 2007. Susceptibility test methods: dilution and disk diffusion methods. In Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller M. A. (Eds). Manual of clinical microbiology, 9th ed, p. 1152–1172. Washington, D.C: ASM Press.

Klaver, F. A. M., Kingma, F. and Weerkamp, A. H. 1993. Growth and survival of bifidobacteria in milk. Netherlands Milk and Dairy Journal 47: 151–164.

Kondamudi, N., Mohapatra, S. K. and Misra, M. 2008. Spent coffee grounds as a versatile source of green energy. Journal of Agricultural and Food Chemistry 56: 11757-11760.

Marhamatizadeh, M. H., Ehsandoost, E. and Gholami. P. 2014. The effect of coffee extract on the growth and viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt. Journal of Food Biosciences and Technology 4: 37–48.

Montella, R., Coïsson, J. D., Travaglia, F., Locatelli, M., Malfa, P., Martelli, A. and Arlorio, M. 2013. Bioactive compounds from hazelnut skin (*Corylus avellana* L.): Effects on Lactobacillus plantarum P17630 and Lactobacillus crispatus P17631. Journal of Functional Food 5: 306–315.

Narita, Y. and Inouye, K. 2011. Inhibitory effects of chlorogenic acids from green coffee beans and cinnamate derivatives on the activity of porcine pancreas α-amylase. Food Chemistry 127: 1532–1539.

Natella, F., Nardini, M., Giannetti, I., Dattilo, C..and Scaccini, C. 2002. Coffee drinking influences plasma antioxidant capacity in humans. Journal of Agricultural and Food Chemistry 50: 6211–6216.

Nikaido, H. 1994. Prevention of drug access to bacterial targets: permeability barrier and active efflux. Science 364: 282–388.

Oke, F., Aslim, B., Ozturk, S. and Altundag, S. 2009. Essential oil composition, antimicrobial and antioxidant activities of Satureja cuneifolia Ten. Food Chemistry 112: 874–879.

Prior, R. L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290–4302.

Radha, K. K., Babuskin, S., Azhagu, S. B. P., Sasikala, M., Sivarajan, M. and Sukumar. M. 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. International Journal of Food Microbiology 171: 32–40.

Ramalakshmi, K., Rao, L. J. M., Takano-Ishikawa, Y. and Goto, M. 2009. Bioactives of low grade green coffee and spent coffee in different in vitro model systems. Food Chemistry 15: 79–85.

Ricardo, N. M. J., Luís, M., Magalhães, M. and Lopes, J. A.. 2013. FT-NIR spectroscopy as a tool for valorization of spent coffee grounds: Application to assessment of antioxidant properties. Food Research International 51: 579–586.

Rufián-Henares, J. A. and de la Cueva, S. P. 2009. Antimicrobial activity of coffee melanoidins — A study of their metal-chelating properties. Journal of Agricultural and Food Chemistry 57: 432–438

Shan, B., Cai, Y. Z., Brooks, J. D. and Corke, H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology 117: 112–119.

Silva, M. A., Nebra, S. A., Silva, M. J. M. and Sanchez, C. G. 1998. The use of biomass residues in the Brazilian soluble coffee industry. Biomass and Bioenergy 14: 457–467.

Singleton, V. and Rossi., J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144–158.

Umemura, M., Fujii, S., Asano, I., Hoshino, H. and Iino, H. 2004. Effect of Coffee mix drink containing mannooligosaccharides from *Coffee mannan* on defecation and fecal microbiota in healthy volunteers. Food Science and Technology Research 10: 195–198.

Vignoli, J. A., Bassoli, D. G. and Benassi, M. T. 2011. Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: the influence of processing conditions and raw material. Food Chemistry 124 :863–868.