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Antimicrobial Activity of Coffee Melanoidins—A Study of Their Metal-Chelating Properties

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Melanoidins comprise a substantial proportion of severely heat-treated foods such as baked cereals or roasted coffee and are widely consumed dietary components. The antimicrobial activity of coffee melanoidins against different pathogenic bacteria has been studied, finding that such activity is due to their metal-chelating properties. Three different mechanisms have been observed: at low concentrations melanoidins exerted a bacteriostatic activity mediated by iron chelation from the culture medium; in the case of bacterial strains that are able to produce siderophores for iron acquisition, melanoidins chelate the siderophore−Fe^{3+} complex, which could decrease the virulence of such pathogenic bacteria; and, finally, coffee melanoidins also exerted a bactericide activity at high concentrations by removing Mg^{2+} cations from the outer membrane, promoting the disruption of the cell membrane and allowing the release of intracellular molecules.

KEYWORDS: Antimicrobial activity; Maillard reaction; melanoidins; metal-chelating activity

INTRODUCTION

Melanoidins are brown high molecular weight compounds formed in the last stage of the Maillard reaction of thermally treated foods (*1*). They are widely distributed in foods and exert different in vitro functional properties such as antioxidant (*2, 3*), antihypertensive (*4*), and metal-binding activities (*5*−*7*) or antimicrobial effects (*8*). The antimicrobial activity of Maillard reaction products (MRP) has been previously studied in coffee (*9*−*11*), but only a few studies have dealt with the antimicrobial activity of isolated coffee melanoidins (*8, 12, 13*). The exact antimicrobial mechanism of MRP is still unclear and depends on the papers consulted. Einarsson and co-workers (*14*) stated that the high molecular weight fraction of model sugar−amino acid systems could develop its antimicrobial action by binding essential metals such as iron. However, Einarsson (*15*) referred in another paper to the antimicrobial activity interference with the uptake of serine, glucose, and oxygen, inhibiting the sugar catabolizing enzymes of microorganisms (*16*) or their potential antioxidant activity (*17*). Only very recently (*12*) has a membrane damage mechanism been proposed as the explanation for the bactericide activity of melanoidins isolated from coffee and biscuits.

The chemical structure of melanoidins has not been completely elucidated yet, although they behave as anionic material (*18, 19*) and can form stable complexes with metal cations (*6, 20*). Hashiba (*21*) reported that the ketone or hydroxyl groups of pyranone or pyridone residues can act as donor groups in melanoidins and participate in the chelation with metals. However, Morales et al. (*5*) found that the chromophoric groups were not the main co-ordination sites for iron complexation in the melanoidin structure.

The results in the literature seem to indicate that the antimicrobial activity of melanoidins could be related to their anionic charge and their ability to chelate some cations such as Fe, Zn, and Cu (*22*), which are essential for the growth and survival of pathogenic bacteria (*14*). In addition, our previous findings (*12*) proved that the anionic behavior of melanoidins allows the disruption of *Escherichia coli* membrane by chelating the stabilizing cation Mg^{2+}. Therefore, the aim of this work was to study if coffee melanoidins could be good candidates as naturally formed antimicrobial agents in thermally processed foods and unravel the relationship between their metal-chelating properties and antimicrobial activity. The effect of melanoidins over cell membrane was studied in different bacterial strains, both Gram-positive and -negative. On the other hand, the effect of iron chelation was checked, as iron is a necessary trace cation for the growth of microorganisms. Finally, the effect of melanoidins over some virulence factors such as siderophores was also studied.

MATERIALS AND METHODS

Chemicals. Chrome-azurol-S complex (CAS), hexadecyltrimethylammonium bromide (HDTMA), piperazine, and 5-sulfosalicylic acid were obtained from Sigma (St. Louis, MO). Ammonium molybdate, nitric acid, perchloric acid, and vanadium pentoxide were from Merck (Darmstadt, Germany) and $FeCl_3 \cdot 6H_2O$ and hydrochloric acid from Panreac (Barcelona, Spain). All glassware and polyethylene materials were washed with tap water after each use, soaked in a 6 N HNO_3 solution (at least overnight), and rinsed several times with bidistilled deionized water to avoid the risk of contamination with iron from other sources.

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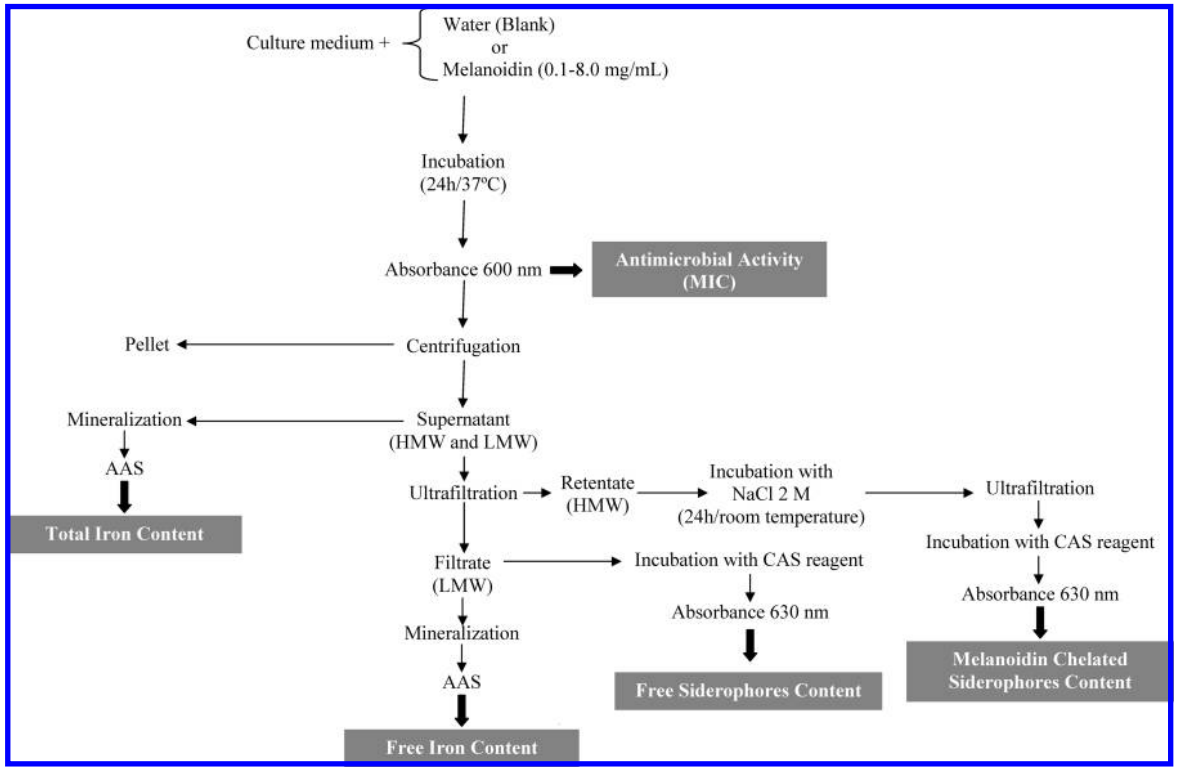

Figure 1. Flow chart for steps performed for the analysis of antimicrobial activity, iron content, and siderophore production.

Microorganisms and Culture Media. The bacterial strains used were from the American Type Culture Collection (ATCC) and included a Gram-positive coccus, *Staphylococcus aureus* ATCC 25923, a Gram-positive rod, *Bacillus cereus* ATCC 11778, and four different Gram-negative bacilli, *Escherichia coli* ATCC 35150, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhymurium* ATCC 13311. In addition, another two different strains that produce siderophores were also assayed: *E. coli* ATCC 33475 and *P. aeruginosa* ATCC 15692. The strains were kept at −70 °C in brain−heart infusion broth (BHI) with 20% glycerol, cultured by transfer into nutritive agar and incubated at 37.0 °C for 24 h. The bacteriological growth medium, BHI, was from Oxoid (Basingstoke, U.K.).

Isolation of Coffee Melanoidins. Filtered coffee brew was produced from a commercial roasted coffee (100% Arabica) supplied by a national producer. Coffee brew was prepared with a mocha type, domestic coffee pot (30 g per 300 mL of water). This brew (150 mL) was diluted with 150 mL of distilled water and filtered (Whatman filter paper no. 40, ashless, Whatman, Maidstone, U.K.). The coffee brew was then subjected to ultrafiltration using an Amicon ultrafiltration stirring cell model 8400 (Millipore, Billerica, MA), equipped with a 10000 Da nominal molecular mass cutoff membrane. The retentate was filled to 300 mL with water and washed again. This washing procedure (diafiltration) was repeated three times. This high molecular weight (HMW) fraction, corresponding to coffee melanoidins, was freeze-dried and stored in a desiccator at 4 °C until analysis.

Assay for Antimicrobial Activity. The antimicrobial activity of water-soluble coffee melanoidins was tested as the minimum inhibitory concentration (MIC) as previously stated by Skyttä and Mattila-Sandholm (*23*) with some minor modifications (*13*). The main steps performed for the antimicrobial activity measurement are depicted in **Figure 1**. Three milliliters of an overnight bacteria culture (10^9 colony-forming units (CFU)/mL) was diluted (1/1000) with fresh BHI broth and incubated at 37.0 ± 1 °C for 3 h to attain exponential phase growth. Bacterial strains, based on their routine growth characteristics, were diluted to optical densities (600 nm) ranging from 0.150 to 0.250. Three hundred microliters of the previous fresh bacteria culture (10^6 CFU/mL) was inoculated into a sterile 96-well microplate (Greiner, Germany). Coffee melanoidins were resuspended in sterile distilled water at different concentrations (0.1−8.0 mg/mL) and filtered by means of a Millipore sterile 0.22 μm filter unit. Subsequently, 50 μL of sample or sterile distilled water (blank assay) was added. Microbial growth was recorded on a FLUOStar microplate reader (BMG Labtech, Bagnols, France). The 96-well microplates were agitated by the microplate reader for 1 min at 150 rpm and then incubated at 37.0 ± 1 °C for 24 h. Turbidity was measured as absorbance at 600 nm and was taken every 5 min. The microplates were shaken for 15 s prior to the measurement to achieve homogeneous suspensions. The MIC was defined as the lowest concentration of coffee melanoidin that did not produce any cell growth (no changes in absorbance values during the entire assay time).

Cell Integrity. The bacterial cell membrane integrity was examined by determination of the release of material absorbing at 260 nm (*24*) as previously tested (*12*).

Iron Determination. The analysis of iron content in culture media was performed by atomic absorption spectroscopy (AAS) as stated by Cabrera et al. (*25*), and the main steps followed are shown in **Figure 1**. Briefly, 300 μL of the samples used for the assay of antimicrobial activity (after the 24 h incubation period) or blanks (mixture of melanoidins and culture media without bacteria) were centrifuged at 11000*g* for 10 min. Samples were then submitted to mineralization for total iron content. However, to measure the free iron content in the culture media, melanoidins were removed by ultrafiltering 250 μL of the supernatant by using Amicon YM-10 Ultrafiltration cones (Millipore) with a 10000 Da molecular weight cutoff at 11000*g* for 30 min. Then, 250 μL of double-distilled water was added to wash melanoidins from any residual iron. Mineralization was initiated by mixing 200 μL of the filtrate, the low molecular weight fraction, with 1 mL of HNO_3 65% (v/v) and a few micrograms of vanadium pentoxide. The mixture was mineralized in a digestion block at 60 °C for 30 min and at 120 °C for 60 min. One milliliter of $HClO_4$ 70% (v/v) was added to the samples, and the mixture was kept at 120 °C for 60 min. The solution was transferred to a glass volumetric flask and diluted with double-distilled deionized water to a volume of 10 mL, and Fe was determined by AAS. All determinations were done in triplicate in a Perkin-Elmer 1100B double-beam atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT).

Siderophore Determination. The assay for siderophore presence was performed according to the method of Schwyn and Neilands (*26*), and the samples were obtained as depicted in **Figure 1**. To measure

the release of siderophores to the culture media, a small amount (200 μL) of the ultrafiltered samples used for the determination of free iron was incubated with the CAS reagent (200 μL) for 1 h at room temperature, and absorbance was read at 630 nm. Blanks were constructed with a mixture of culture media, and the same amount of melanoidin used in the assay for the antimicrobial activity. However, the supernatant obtained for total iron content was used (**Figure 1**) to measure the amount of siderophores chelated by the melanoidins present in the culture media. Two hundred and fifty microliters of supernatant, containing the high molecular weight fraction, was mixed with the same volume of 2 M NaCl and incubated for 24 h at room temperature to release those low molecular weight compounds (LMW) non-covalently attached to the melanoidin core as previously stated (*3, 27*). Siderophores, which were present in the low molecular weight fraction, were harvested after ultrafiltration with Amicon YM-10 cones and quantified with CAS reagent as stated above for the free siderophore assay. In this case, reference solutions (constructed with the ultrafiltrate obtained after incubating a mixture of culture medium−melanoidin and 2M NaCl) were used to avoid any interference from other compounds that are non-covalently attached to melanoidins and different from siderophores.

CAS assay solution was prepared by mixing 6 mL of a 10 mM HDTMA solution, 1.5 mL of 1 mM $FeCl_3 \cdot 6H_2O$ (dissolved in 10 mM HCl), and 7.5 mL of 2 mM aqueous CAS solution slowly added under stirring. Then, 4.307 g of anhydrous piperazine was dissolved and 6.25 mL of 12 M hydrochloric acid was carefully added to a final pH of 5.6, and the volumetric flask was filled with water to afford 100 mL of CAS assay solution. Prior to the reaction, 5-sulfosalicylic acid was added to the above solution at a concentration of 4 mM to obtain the CAS shuttle solution. The solutions were stored in the dark. The color of the CAS complex, ferric iron and HDTMA, changes from blue to orange when iron is removed by siderophores.

Statistical Treatment. All of the analyses were performed at least in triplicate. The Statgraphics v. 5.1 software package was used for statistical analysis. Statistical procedures were performed at a significance level of 95%.

RESULTS AND DISCUSSION

Antimicrobial Activity of Coffee Melanoidins. The activity of coffee melanoidins was measured against eight different bacterial strains, two Gram-positive and six Gram-negative. **Figure 2A** shows a classical dose-dependent inhibition profile of coffee melanoidins over *E. coli* ATCC 35150 (no siderophore production), obtaining similar graphs for the rest of the strains. A decrease of the final absorbance—related with the number of bacteria in the culture medium—was found when the amount of melanoidin added was increased, giving rise to a total inhibition of bacterial growth at the highest melanoidin concentration assayed. This concentration was selected as the MIC for that specific bacterial strain. Bacterial cells were harvested by centrifugation and reincubated again in fresh culture medium without melanoidin, obtaining no bacterial growth after a 24 h incubation period. This confirmed that the antimicrobial activity of coffee melanoidins at such high concentration is bactericidal. A higher melanoidin concentration was necessary to inhibit the microbial growth (**Figure 2B**) in the case of the *E. coli* strain, which is able to produce siderophores (ATCC 33475). This indicates that such a bacterial strain was more resistant to the melanoidin activity. The results for all of the strains studied, expressed as MIC values (**Table 1**), showed that the minimum concentration of melanoidin (with no rise to microbial growth) is between 2.0 and 8.0 mg/mL, these values being in the same range as that previously reported for *E. coli* (*12*). Gram-positive microorganisms are more sensitive to the antimicrobial activity of melanoidins, whereas Gram-negative bacteria had higher MICs. This could be related to the absence in Gram-positive microorganisms of a low-permeability barrier such as the outer membrane, which made them more susceptible to antimicrobial substances (*28*). In this sense, the antimicrobial activity of coffee melanoidins against Gram-positive bacteria was quite similar to that of other polymeric compounds such as chitosan (MIC = 1.25 mg/mL) (*29*). Lastly, is noteworthy to emphasize that those microorganisms with siderophore production capability (*E. coli* ATCC 33475 and *P. aeruginosa* 15692) showed the highest MICs, even higher than the same microorganism but without the capability to produce siderophores (*E. coli* ATCC 35150 and *P. aeruginosa* 27853). Siderophores—considered to be virulence factors—are molecules designed to form tight and stable complexes with ferric iron, allowing the microorganism colonization of adverse media such as the human body (*30*). Therefore, attending to the chelating properties of siderophores against iron, the higher resistance of those bacteria capable of siderophore production could indicate that part of the antimi-

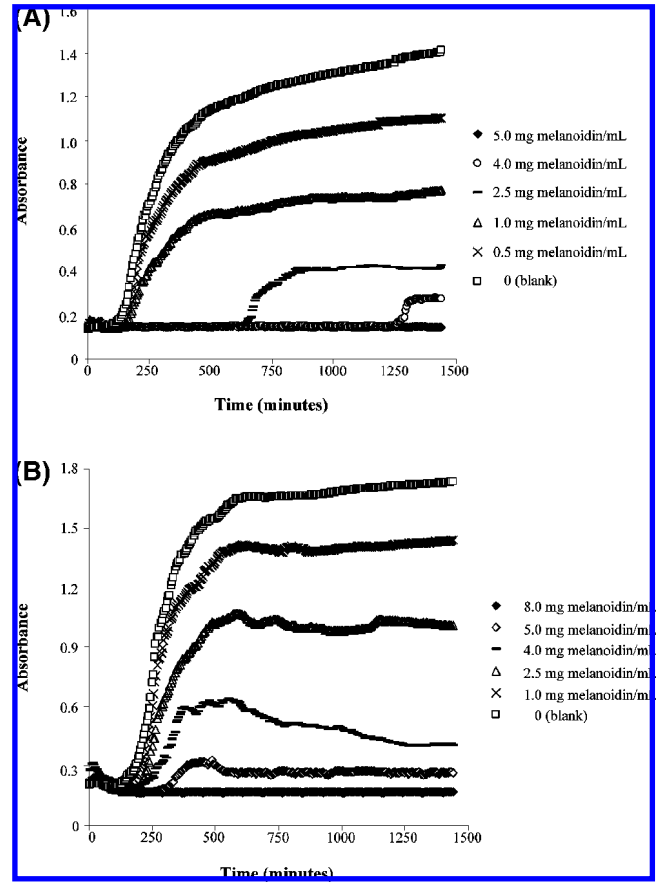

Figure 2. Inhibition of growth for *E. coli* ATCC 35150 (**A**, no siderophore production) and *E. coli* ATCC 33475 (**B**, siderophore production) at different concentrations of coffee melanoidins (0−8 mg/mL) and recorded at 600 nm (37 °C).

Table 1. Minimum Inhibitory Concentrations (MIC) of Coffee Melanoidins

bacteria	strain	shape/ Gram	siderophore production	MIC (mg/mL)
Bacillus cereus	ATCC 11778	rod/G+	−	2.5
Staphylococcus aureus	ATCC 25923	coccus/G+	−	2.0
Proteus mirabilis	ATCC 7002	rod /G−	−	4.5
Pseudomonas aeruginosa	ATCC 27853	rod /G−	−	4.0
Salmonella typhimurium	ATCC 13311	rod /G−	−	4.5
Escherichia coli	ATCC 35150	rod /G−	−	5.0
Pseudomonas aeruginosa	ATCC 15692	rod /G−	+	7.0
Escherichia coli	ATCC 33475	rod /G−	+	8.0

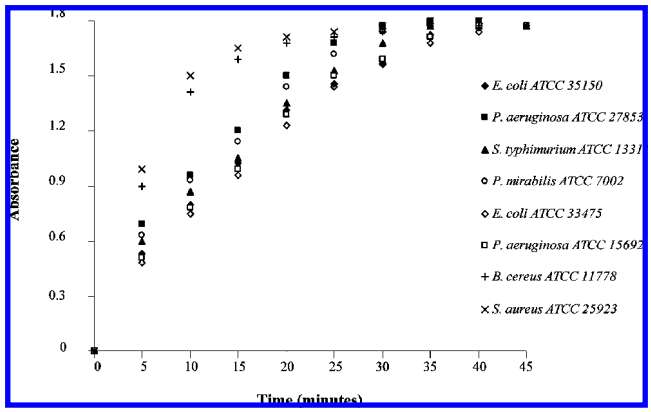

Figure 3. Release of intracellular components at 260 nm of different bacterial strains treated with coffee melanoidins at their minimum inhibitory concentration (MIC).

crobial activity of coffee melanoidins is linked to iron chelation. This point should be checked with other certified strains able to produce siderophores when commercially available.

Cell Integrity. The cytoplasmic cell membrane is the target of many antimicrobial agents, which interact with bacterial membranes, causing disruption and releasing low molecular mass substances (*29*), such as DNA or RNA, that are easily detected by UV at 260 nm as an indication of membrane damage (*31*). Our previous paper (*12*) demonstrated that melanoidins cause *E. coli* membrane disruption by chelating Mg^{2+} ions from the outer membrane, giving rise to a destabilization of the inner membrane. Finally, the release of intracellular compounds ends with cell death. To check this hypothesis in other different bacterial strains, the release of low molecular mass substances was recorded for each kind of bacterium incubated with an amount of melanoidin equal to its MIC. As depicted in **Figure 3**, all of the microorganisms reached the same final absorbance, which means that the final degree of cell damage was similar. However, the speed at which every type of bacterium reached this final damage was different, being faster for Gram-positive and slower for Gram-negatives ones, which had the highest MICs. Gram-negative bacteria have a strong outer membrane where melanoidins bind first and permeabilize (sublethal action). This permeation allows melanoidins to enter the cytoplasmic membrane, where they cause the leakage of cytoplasmic components (the lethal action). However, in the case of Gram-positive bacteria, they are surrounded by a thick coarse peptidoglycan cell wall, which can be rearranged easily by melanoidins. This explains the lower MIC of Gram-positive bacteria and the faster release of cellular compounds.

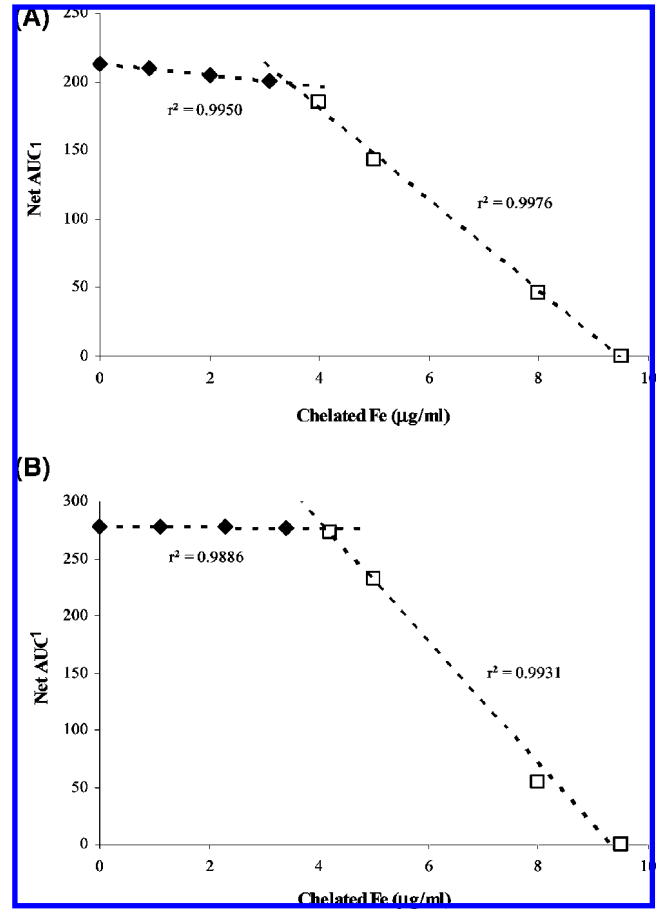

Figure 5. Relationship between chelated iron concentration (μg/mL) and the net area under the curve (AUC) of *E. coli* ATCC 35150 (**A**, no siderophore production) and *E. coli* ATCC 33475 (**B**, siderophore production).

To check this possibility, the experiment was repeated again by adding a high concentration of Mg^{2+} before adding melanoidin. No release of intracellular compounds in Gram-negative bacteria was observed (data not shown), whereas the behavior of Gram-positive ones was unaffected and the same amount of intracellular molecules was measured. This corroborates the idea that the sublethal action of melanoidins is linked to the removal of Mg^{2+} from the outer membrane of Gram-negatives, whereas the lethal action in both Gram-positive and Gram-negative is mediated by other different mechanisms not understood yet. Lastly, using different melanoidin concentrations demonstrated that the cell membrane disruption and release of intracellular

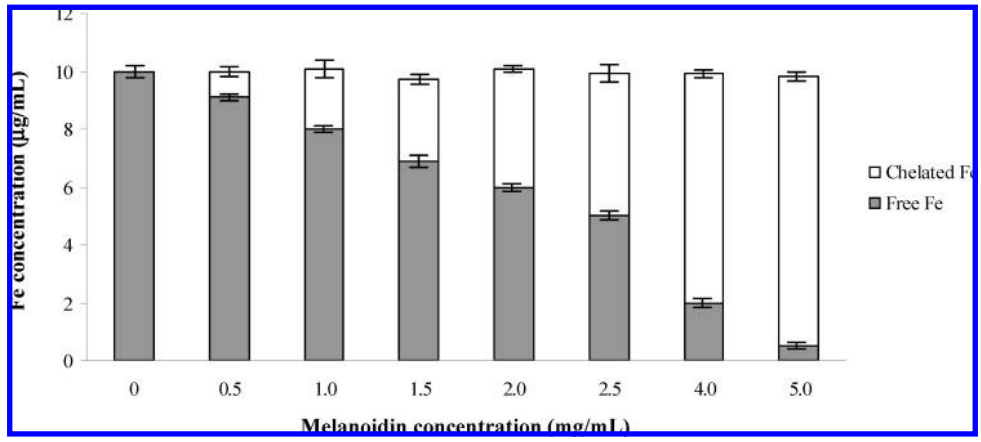

Figure 4. Effect of coffee melanoidins on the amount of free and chelated iron (total amount 10 μg/mL) in the culture medium (BHI broth).

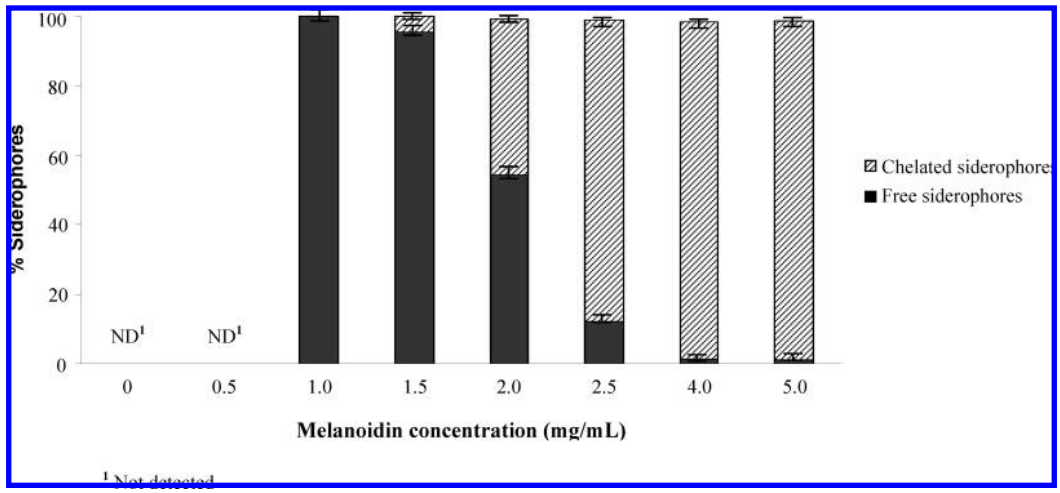

Figure 6. Effect of coffee melanoidins over siderophore concentration in culture media. Siderophore production was measured as absorbance with the CAS reagent. Maximum amount of siderophores was obtained at a melanoidin concentration of 1.0 mg/mL. The absorbance at such point is taken as 100%, the rest of the melanoidin concentrations being referred to that absorbance and expressed as percent.

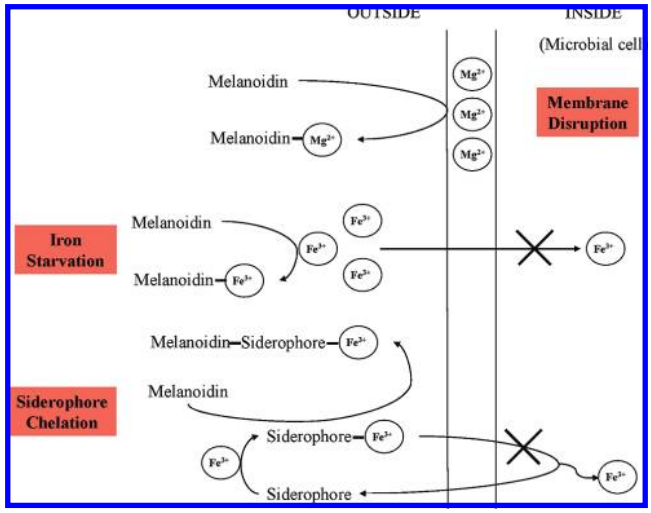

Figure 7. Proposed mechanisms for the antimicrobial activity of coffee melanoidins.

components only started at concentrations very close to the MIC value (data not shown), which indicates that a high critical concentration of melanoidins is necessary to remove Mg^{2+} from the membranes.

The bactericidal activity of melanoidins can be explained by the membrane disruption mechanism stated above, although some authors previously reported (*14*, *16*) that melanoidins and other compounds from the Maillard reaction exerted a bacteriostatic activity over different bacterial strains, increasing the lag phase. Our own investigations (*8*, *33*, *34*) corroborated this hypothesis in the case of isolated melanoidins from other sources at concentrations not higher than 2 mg/mL. At low melanoidin concentrations (up to 1 mg/mL), the microbial growth of *E. coli* ATCC 35150 (no siderophore production) starts at the same time point and the only visible effect is a low final absorbance (**Figure 2A**), which means a lower microbial growth. The final absorbance still decreases for higher melanoidin contents, whereas the starting time point for microbial growth is also affected, increasing for 2.5 and 4.0 mg/mL concentrations. This means that coffee melanoidins exerted a bacteriostatic activity at these melanoidin contents. For the *E. coli* strain capable of producing siderophores (ATCC 33475), the initial starting time point for microbial growth is not affected by melanoidin concentration (**Figure 2B**), although a decrease in the final absorbance was observed. In addition, the final absorbance at each melanoidin content was higher than the one obtained for the strain with no siderophore production. The development of a bacteriostatic activity from melanoidins could be related to iron chelation, taking into account that the main difference between both strains is iron utilization.

Melanoidins are brown anionic polymeric compounds (*18*, *32*) with different charge/mass ratios that depend on the degree of saturation of the reactive groups in the core structure (*19*). This anionic behavior is responsible for the chelating properties of melanoidins over different metals such as iron (*5*) or magnesium (*12*), which finally lead to antimicrobial activity. To get more insight into the bacteriostatic mechanism action of coffee melanoidins, their effect over iron uptake was checked.

Chelating Activity of Coffee Melanoidins against Iron. To study the iron-chelating properties of coffee melanoidins, different melanoidin concentrations were incubated with the culture medium—which showed a mean iron concentration of 10 μg/mL—and the final free iron content was measured (**Figure 4**). The addition of growing amounts of melanoidins produced a decrease of free iron and an increase of chelated iron, which remained linked to the melanoidin core and was not available for bacteria. Fifty percent of the iron was chelated at a melanoidin concentration of 2.5 mg/mL, where the bacteriostatic activity was observed. However, the remaining free iron ranged between 20 and 5% for the different strains at the MICs found (4 and 5 mg/mL). Morales et al. (*5*) calculated that 1 g of coffee melanoidin can bind up to 18.6 g of iron, although they found that their coffee melanoidin bound 50 μg of iron at a concentration of 2.5 mg/mL, which is 10 times higher than our results. However, this difference could be explained by the experimental conditions. On the one hand, these authors performed their experiments at pH 5, whereas the pH of the culture medium was 7.2. This is an important factor because melanoidins become more negatively charged at high pH (*35*) and, therefore, they could bind more cationic species. On the other hand, their experiments were done with an iron solution, whereas in our case the culture medium has different ions competing with iron for coordination sites.

The effect of iron chelation over *E. coli* ATCC 35150 growth (in those with no siderophore production) is depicted in **Figure 5A**. As shown in the figure, there is a lineal relationship between the net area under the curve (net AUC, calculated by subtracting the AUC of the blank sample from the AUC of each melanoidin)

and the chelated iron, indicating that the decrease in bacterial growth is mediated by iron starvation. The chelation of iron has little effect up to a melanoidin concentration of 1.5 mg/mL, where 31% of the total iron was chelated. From this concentration, the increase in the slope of the curve indicates that iron chelation has a deep effect over bacterial growth, suggesting the beginning of the bacteriostatic activity. In this sense, the inflection point between both curves allows the calculation of the amount of chelated iron (or melanoidin concentration) where the bacteriostatic activity starts, corresponding in the case of *E. coli* ATCC 35150 to 3.55 μg of iron chelated or 1.75 mg of coffee melanoidin per milliliter of culture medium. Similar results were obtained for the other bacterial strains with no production of siderophores (data not shown).

The acquisition of iron is possibly the major determinant as to whether a microorganism within an animal is able to maintain itself therein (*36*). Without this ability, it would be unable to grow and will effectively be eliminated by direct attack from the host defense mechanisms or would die of nutrient starvation. Therefore, the acquisition of iron is recognized as one of the key steps in the development of any pathogen in its host. Siderophores, which are specific Fe^{3+}-binding agents, are produced by many but not all microorganisms in response to a deficiency of iron (*30*). The effect of iron chelation over net AUC of a siderophore producer bacterium (*E. coli* ATCC 33475) is shown in **Figure 5B**. As stated above, in the case of the non-siderophore producer *E. coli*, a lineal relationship between net AUC and chelated iron was found. At low melanoidin concentrations, iron chelation has little effect (low curve slope) on bacterial growth up to a melanoidin concentration of 2 mg/mL (42% of iron chelated). This could be related to the strong binding power of siderophores for iron—dissociation constant values ranging from 10^{22} to 10^{50} (*37*)—which is regarded as sufficiently strong for the siderophore to remove iron attached to molecules such as ferritin, probably able to remove iron from melanoidins.

A second curve with a more pronounced slope was also found, indicating a deep effect of iron starvation on microbial growth. In this case, because no bacteriostatic activity was observed in the dose—response plot (**Figure 2B**), another different effect involving siderophores must be present, which is the final responsible molecules that provide iron to these microorganisms. A second set of experiments was performed to study the effect of coffee melanoidins on the concentration of siderophores in the culture medium (**Figure 6**). Siderophore production was initiated when cells were grown with a deficiency of iron of 23%, corresponding to a melanoidin concentration of 1.0 mg/mL. At a concentration of 1.5 mg/mL, there was a decrease (although not statistically significant) of the percent of free siderophores, whereas such a decrease was statistically significant ($P < 0.05$) from a concentration of 2.0 mg/mL. This concentration corresponds to the inflection point that was observed in **Figure 5B**. In addition, with the aid of a high ionic strength, siderophores were removed from the melanoidin core, indicating that they are chelated by melanoidins at high concentrations by non-covalent interactions. The maximum chelating activity was obtained at a melanoidin concentration of 5.0 mg/mL, at which 97.2% of the siderophores were chelated. These results are in line with those previously published by other authors, where coffee melanoidins showed remarkable binding properties toward low molecular weight substances in the food matrix such as furfural or pyrrole derivatives (*38*), chlorogenic acid (*39*), or other phenol-like compounds (*18*).

Melanoidins comprise a substantial proportion, up to 35%, of several foods such as baked cereals or roasted coffee and are widely consumed dietary components. The antimicrobial activity of coffee melanoidins against different bacterial strains has been studied, finding that it is mediated by the metal-chelating properties of melanoidins. Three different mechanisms have been observed and are reviewed in **Figure 7**. At low concentrations, melanoidins exert a bacteriostatic activity mediated by the chelation of iron from the culture medium, although the sequestering of other essential cations cannot be ruled out. In addition, for those bacterial strains able to produce siderophores for iron acquisition, the chelation of the siderophore—Fe^{3+} complex by melanoidins has been demonstrated, which could decrease the virulence of such pathogenic bacteria. Finally, coffee melanoidins also exerted a bactericidal activity at high concentrations by removing Mg^{2+} cations from the outer membrane, promoting the disruption of the cell membrane and allowing the release of intracellular molecules. These results reinforce the idea that water-soluble melanoidins may be good candidates as naturally formed antimicrobial agents in thermally processed foods.

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