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Antimicrobial Activity of Melanoidins against *Escherichia coli* Is Mediated by a Membrane-Damage Mechanism

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Melanoidins are brown polymeric material formed during thermal processing of food and widely distributed in the Western diet. Three water-soluble fractions were isolated from both commercial coffee and biscuit by sequential ultrafiltration steps (3 and 10 kDa cutoff). Biscuits were enzymatically digested to solubilize the protein-linked melanoidin fraction. Antimicrobial activity of melanoidins was evaluated against a Gram-negative reference pathogenic bacterium (*Escherichia coli*). The high-molecular-weight fraction of water-soluble melanoidins (>10 kDa) exerted the highest antimicrobial activity. The mechanism of action was further investigated by cell integrity and outer- and inner-membrane permeabilization assays. At the minimum inhibitory concentration, melanoidins provoked irreversible cell membrane disruption, which was independent of the bacterial transmembrane potential. Results indicate that water-soluble melanoidins killed pathogenic bacteria strains (*E. coli*) by causing irreversible changes in both the inner and outer membranes. Likely, it allows for interference with biosynthetic processes, such as the inhibition of nutrient transport and macromolecular precursors.

KEYWORDS: Antimicrobial activity; Maillard reaction; melanoidins; membrane permeation

INTRODUCTION

The Maillard reaction, occurred in thermally treated foods, takes place between the amino group of a free or protein-bound amino acid and reducing sugars (*1*). Many different Maillard reaction products (MRPs) can be formed depending upon the reaction conditions (e.g., heating time and temperature), physicochemical properties of the system (e.g., water activity or pH), chemical composition, and nature of reagents (*1–3*). The high-molecular-weight compounds formed in the last stage of the Maillard reaction are called melanoidins. Melanoidins are widely distributed in foods and could exert different *in vitro* functional properties, such as antioxidant (*4, 5*), antihypertensive (*6*), metal-binding activity (*7*), antimicrobial (*8*), and prebiotic effects (*9*). In recent years, several studies have been mainly focused in the effect of melanoidins on the human diet and their possible nutritional, biological, and health implications (*10, 11*).

The chemical structure of melanoidins has not been completely elucidated yet, although their investigation has been increased in the present decade. In particular, it has been shown that they can have a different structure according to the different starting material and behave as anionic material (*12–14*): in some cases, they are mainly formed by a carbohydrate skeleton with few unsaturated rings and a small nitrogen component, and in other cases, they can have a protein structure linked to small chromophores (named melanoproteins) or to phenolic residues (*15, 16*).

Antimicrobial activity of MRPs has been previously studied in model systems (*2, 16–19*) and coffee (*20–22*), but specific studies on antimicrobial activity of melanoidins are scarce (*8*). Einarsson and co-workers (*2*) measured the antimicrobial activity of arginine−xilose and histidine−glucose Maillard reaction mixtures. They fractionated the model systems by dialysis (molecular cutoff at 1000 Da) and found that the high-molecular-weight fraction exerted a higher antimicrobial activity than the low-molecular-weight one. The authors stated that the high-molecular-weight fraction (melanoidins principally) could develop its antimicrobial action by binding essential metals, such as iron, which is essential for growth and survival of pathogenic bacteria. In addition, other authors referred the antimicrobial activity of Maillard reaction compounds to the interference with the uptake of serine, glucose, and oxygen (*18*), to inhibit the sugar catabolizing enzymes of microorganisms (*17*) or their potential antioxidant activity (*23*). However, the exact mechanism by which melanoidins or MRPs affect the bacteria growth is not known.

Therefore, the aim of this study was to investigate the antimicrobial activity and elucidate an antimicrobial mechanism of water-soluble melanoidins and MRPs obtained from coffee and biscuits. The activity is evaluated over *Escherichia coli* as

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Gram-negative model bacteria because these kinds of bacteria are more resistant to antibiotics and toxic drugs because of the presence of an outer membrane (*24*).

MATERIALS AND METHODS

Chemicals. *O*-Nitrophenyl-L-D-galactoside (ONPG), 1-*N*-phenylnaphthylamine (NPN), and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were obtained from Sigma (St. Louis, MO), and dichloromethane was obtained from Panreac (Barcelona, Spain). Bacteriological growth media, brain heart infusion broth (BHI), and lactose broth (LB) were from Oxoid (Basingstoke, U.K.). The bacterial strain used for the antimicrobial assay was *E. coli* American Type Culture Collection (ATCC) 11775. The assay organisms were stored frozen at −80 °C in medium containing 25% glycerol. All other reagents were of the highest grade available commercially. Filtered coffee brew was produced from commercial roasted coffee (blend of 80% Arabica and 20% Robusta) supplied by a national producer. Coffee brew was lyophilized and stored at −20 °C until analysis. A commercial breakfast biscuit was purchased from a local store.

Isolation of Coffee Fractions. A total of 1 g of the lyophilized coffee brew was resuspended in 100 mL of hot water (50–60 °C) for 3 min under continuously stirring. The coffee brew obtained was then filtered (Whatman Filter Paper number 40, ashless, Whatman, U.K.) and defatted with dichloromethane (2 × 200 mL). The coffee brew was then subjected to ultrafiltration using an Amicon ultrafiltration cell model 8400 (Amicon, Beverly, MA), equipped with a 10 000 Da nominal molecular mass cutoff membrane. The retentate was filled up to 200 mL with water and washed again. This washing procedure (diafiltration) was repeated at least 3 times. The high-molecular-weight (HMW) fraction corresponding to melanoidins (RC10 sample; retentate of coffee at 10 kDa) was freeze-dried and stored in a desiccator at 4 °C until analysis. The eluates obtained after 10 000 Da ultrafiltration were mixed and ultrafiltered against a 3000 Da nominal molecular mass cutoff membrane. Then, two fractions were obtained, a retentate (RC3; retentate of coffee at 3 kDa) with an intermediate-molecular-weight compounds and a filtrate (FC3; filtrate of coffee at 3 kDa) with the low-molecular-weight (LMW) compounds. These two fractions were also lyophilized and stored at 4 °C until analysis.

Isolation of Biscuit Fractions. Biscuit melanoidins and related fractions were prepared in a similar way than coffee ones, although a sample solubilization was carried out as described by Borrelli et al. (*10*) with some modifications. Briefly, homogenized biscuit samples (500 mg) were diluted in 25 mL of a Pronase-E solution containing 0.375 mg of Pronase E (7.5 units/mg) in 0.1 M sodium−borate at pH 8.2 buffer, vigorously stirred, and incubated at 37 °C for 40 h under shaking. The reaction was stopped by cooling in an ice-water bath followed by addition on 100 μL of trichloroacetic acid solution (40%, w/v) and centrifugation at 4500 g/10 min at 4 °C. The supernatants were filtered (0.45 μm) and stored at −20 °C until analyzed. A sequential ultrafiltration procedure was applied as reported for coffee. Subsequently, three fractions were obtained; RB10, RB3, and FB3, corresponding to the fractions higher than 10 kDa, between 3 and 10 kDa, and lower than 3 kDa, respectively.

Assay for Antimicrobial Activity. The antimicrobial activity of water-soluble coffee and biscuit fractions was investigated against a Gram-negative bacteria (*E. coli*) purchased from ATCC. The minimum inhibitory concentration (MIC) was tested as stated previously by Skyttä and Mattila-Sandholm (*25*) with some minor modifications. A total of 250 μL of a fresh bacteria culture (10^6 colonies/mL) grown in BHI were inoculated into a sterile 96-well microplate (Greiner, Germany). Coffee and biscuits fractions were resuspended in sterile distilled water at different concentrations (2.5–50.0 mg/mL) and filtered by means of a Millipore sterile 0.22 μm filter unit previously used. Subsequently, 50 μL of sample or sterile distilled water (blank assay) were added. Microbial growth was recorded on a Synergy-HT multidetector microplate reader driven by Gen5 reader control and data analysis software (BioTek Instruments, VT). 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 10 s prior to the measurement to achieve homogeneous suspensions. The MIC was defined as the lowest concentration of food fraction at which the cell growth was not recorded (no changes in absorbance values during the entire assay time).

The study of the bactericide−bacteriostatic activity was performed as stated above, but in this case, the mixtures sample−culture medium were incubated in sterile 96-well microplates for only 3 h. At the end of the incubation, cells were harvested by centrifugation at 11000*g* for 10 min, washed, and resuspended in 20 mM phosphate buffer (pH 7.0). Then, serial decimal dilutions were prepared using the 20 mM phosphate buffer, and 20 μL aliquots of these dilutions were spread onto fresh BHI agar plates. The number of CFU was determined after incubation at 37 °C for 24 h. Analyses were made in triplicate.

Cell Integrity. The bacterial cell membrane integrity was examined by determination of the release of material absorbing at 260 nm (*26*). Bacterial cultures grown were harvested by centrifugation at 11000*g* for 10 min, washed, and resuspended in 0.5% NaCl solution. The final cell suspension was adjusted to an absorbance of 0.7 at 420 nm. A 1.5 mL portion of derivative solution was mixed with 1.5 mL of bacterial cell suspension, and the release over time of materials absorbing at 260 nm was monitored with a Shimadzu UV–vis 1603 (Duisburg, Germany) spectrophotometer using a 1 cm path-length cell.

Outer-Membrane (OM) Permeabilization Assay. The OM permeabilization activity was determined by the NPN assay described by Ibrahim et al. (*26*). *E. coli* cultures grown in LB medium were harvested by centrifugation at 11000*g* for 10 min, washed, and resuspended in 0.5% NaCl solution. The final cell suspension was adjusted to obtain an absorbance at 420 nm of 1.00. In a quartz cuvette, 20 μL of 1 mM NPN was added to a 1 mL volume of bacteria. The background fluorescence was recorded using a SMF-25 fluorescence spectrophotometer (Kontron Instruments, Milan, Italy) with 1 cm path-length cuvettes (QS-1.000 Suprasil, Hellma GmbH and Co, DE). Excitation and emission wavelengths were set at 350 and 429 nm, respectively. Aliquots of coffee and biscuit fractions were added. An increase in fluorescence because of the partitioning of NPN into the OM was recorded as a function of time until no further increase in intensity. Control tests performed by the addition of double-distilled water as a blank were performed to verify that the enhanced fluorescence was due to NPN uptake by bacteria.

In testing the effect of $MgCl_2$ to the fraction-induced fluorescence, this salt was added to the buffer before NPN.

Inner-Membrane (IM) Permeabilization Assay. IM permeabilization was determined by measuring the release of cytoplasmic β-galactosidase activity from *E. coli* into the culture medium using ONPG as the substrate (*26*). Logarithmic-phase bacteria grown in nutrient broth containing 2% lactose were harvested, washed, and resuspended in 0.5% NaCl solution. The final cell suspension was adjusted to obtain an absorbance reading of 1.20 at 420 nm. The bacterial suspension (200 μL) was pipetted into the wells of the microtiter plate, and 10 μL of ONPG (30 mM) and aliquots of coffee and biscuit fractions were added to each well. Plates were incubated with gentle rocking at 37 °C, and the production of *o*-nitrophenol over time was monitored at 420 nm. In certain experiments, 4 μL of CCCP stock was added a few minutes before the peptides to a final concentration of 100 μM. To distinguish between the release of the cytoplasmic enzyme and ONPG uptake into cells, the bacteria were removed by centrifuge after 60 min of incubation with the peptide and the release of β-galactosidase in the medium supernatant was measured. The release was quantified as the production of *o*-nitrophenol (ONP) per minute per milliliter and calculated using the following formula: $[A_{420}/\text{sample volume } (\mu L)]/\text{reaction time (min)} \times 4.86$, where A_{420} and 4.86 were the absorbance at 420 after a certain time of enzyme reaction and the extinction coefficient (in mM^{-1} cm^{-1}) of ONP, respectively.

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

Table 1. MIC[a] Values of Coffee and Biscuit Melanoidin Fractions against *E. coli*[b]

	fraction	MIC value (mg/mL)
coffee	RC10	10.0
	RC3	25.0
	FC3	45.0
biscuit	RB10	7.5
	RB3	20.0
	FB3	50.0

[a] MIC = minimum inhibitory concentration. [b] Codes for fractions are described in the Materials and Methods.

RESULTS AND DISCUSION

Antimicrobial Activity of Coffee and Biscuit Water-Soluble Fractions. Three fractions with different molecular weight (>10, between 10 and 3, and <3 kDa) were obtained from roasted coffee and a classical breakfast biscuit. The high-molecular-weight fraction of coffee (RC10) covered about 19.1% of the total coffee brew dry mass, where RC3 and FC3 accounted for 17.6 and 63.3%, respectively. In contrast, a previous enzymatic treatment is necessary to extract the material from cookies. After enzymatic digestion, about 43% of the initial cookie dry mass was soluble, corresponding to 1.9, 9.0, and 32% of the RB10, RC3, and FB3 fractions, respectively.

Antimicrobial activities of former fractions were evaluated using MIC values against a Gram-negative bacterium, *E. coli* (**Table 1**). Among the fractions, the most intense antimicrobial activity was obtained for the 10 kDa fractions (10.0 and 7.5 μg mL^{-1} for coffee and biscuits, respectively), whereas up to 5 times lower activity was found for the LMW fraction; in the case of the 10–3 kDa fractions, their antimicrobial activity was intermediate to that of the above-reported fractions. In this sense, the antimicrobial activity of HMW compounds is weak if compared to natural LMW compounds, such as carvacrol, cinnamaldehyde, or thymol (MIC values comprised between 0.30 and 0.66 mg/mL) (*27, 28*), but it is quite relevant if compared to the antimicrobial activity of other natural HMW compounds, such as chitosan (MIC of 1.25 mg/mL) (*29*).

On the other hand, the high- or intermediate-molecular-weight (IMW) compounds of biscuits exerted a slightly higher antimicrobial activity compared to the ones obtained for the coffee ones. On the contrary, the LMW fraction of coffee showed a higher activity when compared to that of biscuits. This could be a consequence of the most intense thermal treatment applied to coffee, which gives rise to the generation of most LMW compounds with potential antimicrobial activity (such as heterocyclic amines, furans, etc.) (*20*).

Some authors reported previously (*2, 17*) that melanoidins and other compounds from the Maillard reaction exerted a bacteriostatic activity over different bacterial strains, increasing the lag phase. Our own investigation (*8*) corroborated this hypothesis in the case of isolated melanoidins from other sources at concentrations not higher than 2 mg/L. To evaluate if the antibacterial activity obtained for every fraction was bacteriostatic or bactericide, mixtures of sample−culture medium were incubated for 3 h. After this period, cells were harvested by centrifugation (to eliminate the active compounds present in the culture medium) and incubated for 24 h in BHI agar plates and, finally, the number of CFU was determined. It was found (**Table 2**) that the HMW fractions exerted a bacteriostatic activity at low concentrations (2.5 mg/mL), whereas this bacteriostatic activity was observed at higher concentrations for the IMW and LMW fractions (up to 30–35 mg/mL for LMW). In addition, a bactericide activity was obtained when the concentration raised some limits (5.0 mg/mL for HMW, 10–15 mg/mL for intermediate compounds, and 35–40 mg/mL for LMW). At the MIC concentrations, *E. coli* cells could not grown in the culture media, indicating that all of the cells were killed. These results support the hypothesis that melanoidins damage cells in an irreversible way that inhibits their growth as well as if cells are exposed to melanoidins for a short period of time and then removed from the culture media.

Melanoidins, which are the main constituents of the HMW fraction, are brown anionic polymeric compounds (*12, 13*) with different charge/mass ratios that depend upon the degree of saturation of the reactive groups in the core structure (*14*). In addition, melanoidins can bind essential metals, such as iron (*7*). In this sense, some substances with anionic behavior and chelating properties, such as ethylenediaminetetraacetic acid (EDTA) (*30*), have antimicrobial activity because of the profound effect on the OM permeability barrier of Gram-negative enteric bacteria. It removes, by chelation, stabilizing divalent cations from their binding sites in LPS, resulting under certain conditions in the rupture of the OM. To obtain more insight into the mechanism action of melanoidins over *E. coli*, the effect of the isolated fractions over the cell membrane by specific assays was checked.

Cell Integrity. The cytoplasmic cell membrane is the target of many antimicrobial agents, which interact with bacterial membranes causing changes in the bacterial membrane (*29*). When bacterial membranes become compromised, low-molecular-mass substances followed by macromolecules are left out. These intracellular components, such as DNA or RNA, are easily detected by UV at 260 nm as an indication of membrane damage (*31*).

The release of intracellular components from *E. coli* treated with coffee or biscuit fractions is shown in **Figure 1**. The absorbance at 260 nm was increased in a time-dependent manner because of the addition of the different fractions. The release of intracellular components with the HMW fraction (RB10 and RC10) was higher than that of IMW fractions (RB3 and RC3), whereas slight changes were obtained for the LMW extracts. There was a dramatic increase up to 40 min; thereafter, the absorbance was almost unchanged. These results indicate that the cell-membrane disruption is faster for melanoidins, which correlates with their low MIC value.

OM Permeabilization of *E. coli*. Fluorescence of the NPN probe increases when incorporated into the hydrophobic core of a Gram-negative bacterial cell membrane (after permeation) compared to the fluorescence of a nonpermeating bacterial cell. On the basis of this principle, the OM permeabilization of live *E. coli* by the different fractions was monitored using the hydrophobic NPN fluorescent probe. As shown in parts **A** and **B** of **Figure 2**, the addition of high- or intermediate-molecular-weight fraction *E. coli* suspensions in the presence of NPN caused an increase in fluorescence, which indicates that *E. coli* cell membranes were damaged by these fractions. In a general way, the OM permeabilization exerted by the HMW fraction was faster and almost doubled the activity of the IMW fractions. This result also strengthens the fact, previously stated for the release of intracellular substances, that melanoidins quickly disrupts cell membranes of bacteria more than the intermediate fractions. Moreover, it was observed that the activity of RB10 was one-third higher than the one of RC10.

The OM of Gram-negative bacteria consists of lipopolysaccharides (LPSs) and proteins, and these are maintained together by electrostatic interactions with divalent cations required to stabilize the OM. Polycationic molecules, such as chitosan, could

Table 2. Inhibitory Effect of Coffee and Biscuit Fractions (Expressed as a Percentage of Survival ± Standard Deviation) against *E. coli*[a]

	concentration (mg/mL)											
fraction	2.5	5.0	7.5	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0
RC10	100 ± 0	87 ± 9	37 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
RC3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	76 ± 4	32 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
FC3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	67 ± 6	34 ± 5	0 ± 0	0 ± 0
RB10	100 ± 0	42 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
RB3	100 ± 0	100 ± 0	100 ± 0	62 ± 5	31 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
FB3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	74 ± 3	26 ± 2	0 ± 0

[a] Codes for fractions are described in the Materials and Methods.

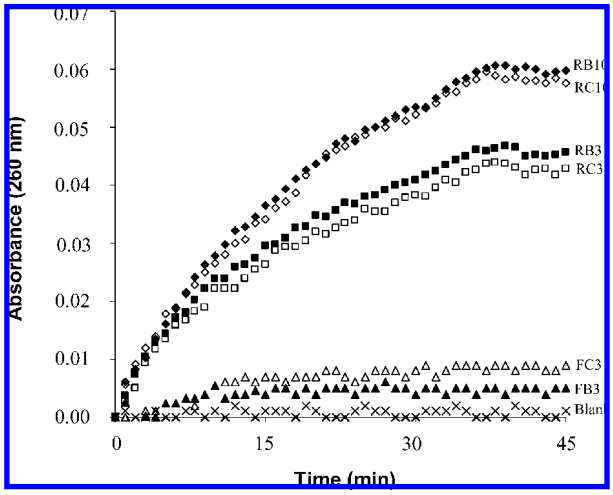

Figure 1. Effect of different coffee and biscuit fractions (10 mg/mL) on the cell integrity measured as a release of intracellular components at 260 nm.

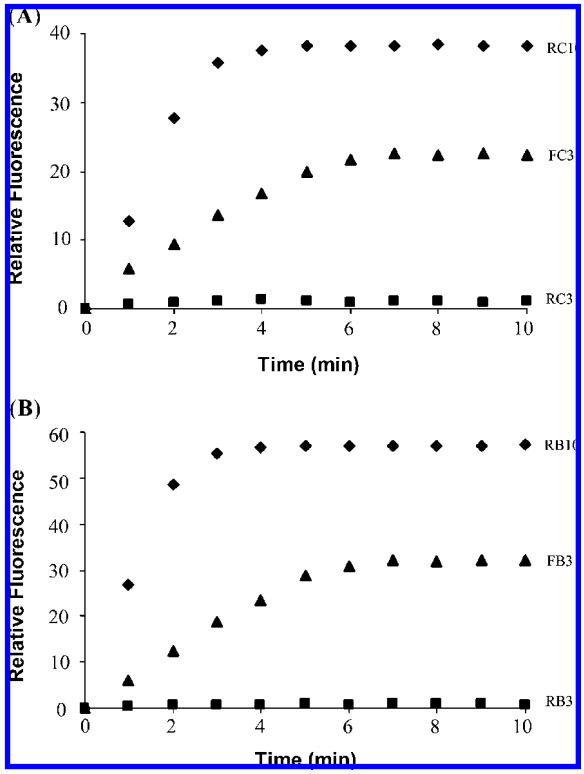

Figure 2. Effect of different coffee (A) and biscuit (B) fractions (10 mg/mL) on the outer-membrane permeabilization of *E. coli* as measured by the uptake of 1-*N*-phenyl-naphthylamine (NPN).

bind to the negatively charged O-specific oligosaccharide units of *E. coli* LPSs (*29*). However, anionic substances, such as EDTA, remove by chelation stabilizing divalent cations from their binding sites in LPS (*31*) and thus disrupting the integrity of the OM and resulting in the loss of the barrier function or blocking of the nutrient flow with concomitant bacterial death because of depletion of the nutrients. It could be hypothesized that melanoidins, because of their negative surface charge (*12, 14*), could bind to the OM by electrostatic interactions and chelate Mg^{2+}, which is a divalent cation that plays a specific structural role, stabilizing the prokaryotic membranes as metal ion bridges between phosphate groups of phospholipids (*26*). To check this hypothesis, the effect of $MgCl_2$ addition over the uptake of NPN by *E. coli* treated with the different fractions at a concentration of 10 mg/mL was assayed. The addition of $MgCl_2$ produced a strong decrease on the fluorescence production, indicating the interaction of melanoidins and the compounds isolated in the intermediate fraction with Mg^{2+} as shown in **Table 3**, which corroborates the above-mentioned hypothesis.

Table 3. Effect of $MgCl_2$ Addition over the Uptake of NPN[a] by *E. coli* Treated with 10 mg/mL of Coffee or Biscuit Melanoidin Fractions[b]

		$MgCl_2$	
	fraction	−	+
coffee	RC10	38.2 ± 3.1	8.9 ± 1.0
	RC3	22.4 ± 1.3	3.8 ± 0.5
	FC3	1.1 ± 0.2	0.9 ± 0.3
biscuit	RB10	57.2 ± 2.4	10.8 ± 0.9
	RB3	32.1 ± 1.4	4.6 ± 0.6
	FB3	0.8 ± 0.4	0.6 ± 0.2

[a] NPN = 1-*N*-phenyl-naphthylamine. [b] Results are expressed as a percentage of fluorescence intensity ± standard deviation. Codes for fractions are described in the Materials and Methods.

IM Permeabilization of *E. coli*. Destabilization of the OM is necessary to gain access to the IM. Because of OM permeation was demonstrated, the HMW and IMW fractions could interact with the IM. To study this possible effect, the IM permeation was evaluated as a function of cytoplasmic β-galactosidase release, with bacteria grown in lactose-containing medium. When cells were treated with a 10 mg/mL concentration of each fraction (**Figure 3A**), a lag time of about 35 min was followed by a progressive release of the cytoplasmic β-galactosidase in the case of FG3 and FC3. However, in the case of the HMW fractions, the release of β-galactosidase started immediately, reaching a steady state at 70 min. This behavior could be explained taking into account that the HMW fractions were present in the culture media at bactericide concentrations, whereas IMW compounds were not. In addition, the highest β-galactosidase release by RB10 corroborates its most intense activity against *E. coli*. To obtain more insight into the permeation activity of HMW fractions, they were used to follow the study because of their strong effect over IM permeation.

CCCP is an uncoupler of oxidative phosphorilation in mitochondria, being capable of depolarizing both plasmatic and mitochondria membranes, and then, it can inhibit the IM

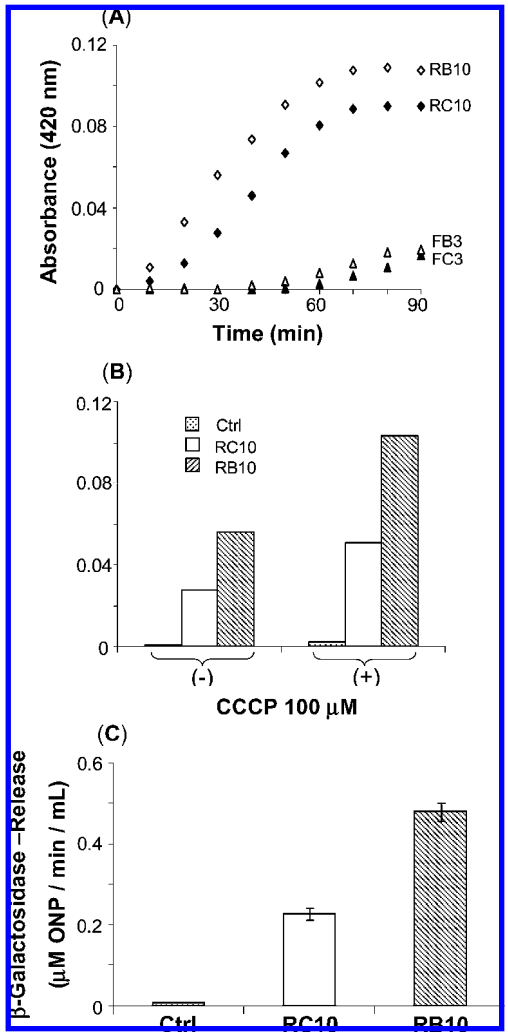

Figure 3. Effect of different coffee and biscuit fractions on the inner-membrane cell integrity as measured by the release of cytoplasmic β-galactosidase activity of *E. coli*. (A) Time dependence of the permeation of cells treated with 10 mg/mL of coffee or biscuit fractions. (B) Bacteria treated with 10 mg/mL of RC10 or RB10 for 30 min in the absence or presence of uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). (C) Bacteria (after 60 min of incubation) were removed by centrifugation, and enzyme release was assayed in the cell-free supernatant. Ctrl, cells treated with saline alone. Codes for fractions are described in the Materials and Methods.

permeation of those species that based its activity in the transmembrane potential. As seen in **Figure 3B**, pretreatment of *E. coli* with 100 μM CCCP resulted in a 2-fold increase of fraction-mediated IM permeation, suggesting that membrane disruption is independent of the bacterial transmembrane potential. To see whether the effect of the HMW fractions on IM increased the release of the cytoplasmic enzyme or membrane lesions allowing ONPG uptake into cells, the specific activity of β-galactosidase released in the cell-free medium was measured (**Figure 3C**). HMW fractions caused considerable release of the enzyme into the medium within 60 min of incubation with cells, being double for the biscuit HMW fraction. This result demonstrates that melanoidins disintegrate the bacterial membranes and might allow for interference with biosynthetic processes occurring at the membrane, which are important in bacterial viability, such as inhibition of the transport of nutrient and macromolecular precursors.

Melanoidins comprise a substantial proportion of several foods, such as baked cereals or roasted coffee and malt, and are widely consumed dietary components. The antimicrobial activity against *E. coli* of three coffee or biscuit fractions (differing in molecular weight) has been studied. The highest activity has been obtained for the high-molecular-weight fraction of melanoidins, being stronger for biscuit melanoidins. Antimicrobial activity has been proven to be bacteriostatic at low concentrations but bactericide at the higher ones. In addition, it has been demonstrated that melanoidins killed bacteria by disrupting both the outer and inner membranes of *E. coli*. These results suggested that water-soluble melanoidins may be good candidates as naturally formed antimicrobial agents in thermally processed foods. However, the metabolic fate of melanoidins is still obscure, although experiments *in vitro* and in animals show that they are susceptible to be metabolized by the microflora present in the hingut and subsequently to influence intestinal health.

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