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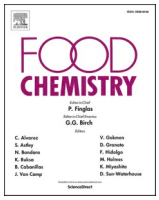

Short communication

Antimicrobial properties and volatile profile of bread and biscuits melanoidins

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ABSTRACT

This work gives novel information about the antimicrobial effect and volatiles of melanoidins isolated from Maria biscuit, common and soft bread. Melanoidins were isolated from scraped and sieved crusts (1 mm), after gluten digestion, 10 kDa ultrafiltration, and diafiltration. Finally, they were freeze-dried. Headspace solid-phase dynamic extraction coupled with a gas chromatograph with a mass spectrometer was used to determine the volatile profiles. The antimicrobial effect was evaluated against isolated strains of the most relevant food spoilage and pathogen microorganisms, together with some molds and yeasts. Melanoidins from common bread exhibited the most extensive antimicrobial activities and showed the most composite volatile profile. No undesirable compounds, such as furfural and 5-hydroxy-methyl-furfural, were found in any of the melanoidins studied. The obtained data pointed out that bakery melanoidins can exert effective food technological properties as natural antimicrobials that can improve shelf-life and security of foodstuffs, together with a possible contribution to food aroma.

1. Introduction

Melanoidins are heterogeneous, brown-coloured compounds containing nitrogen, with high molecular weight (HMW), produced in the last stages of the Maillard reaction that takes place during the heating treatment of foods (Wang, Qian & Yao, 2011). Melanoidins structure is influenced by the raw material, and the processing conditions (Kanzler & Haase, 2020; Pastoriza & Rufián-Henares, 2014; Tagliazucchi & Bellesia, 2015; Borrelli & Fogliano, 2005) then their unit structure is unspecific. Bread melanoidins are colored HMW compounds (>10 kDa) called melanoproteins because are mainly formed by gluten proteins, cross-linked with different colored Maillard reaction products. Protein-bound pyrrolinone reductonyl-lysine, also known as pronyl-L-lysine, has been identified in bread crust (Lindenmeier, Faist & Hofmann, 2002). Bakery melanoidins can be efficiently extracted after enzymatic digestion (Borrelli & Fogliano, 2005; Walker et al., 2020), together with

Abbreviations: BM, melanoidins isolated from Marie biscuit; CBM, melanoidins isolated from common bread; CFU, colony forming units; EB, enterobacteriaceae; LAB, lactic acid bacteria; M&Y, molds and yeasts; o/n, one night; PS, *Pseudomonas* spp.; SBM, melanoidins isolated from soft bread; TAM, total anaerobic mesophilic microorganisms.

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other isolation and purification techniques as dialysis, ultrafiltration, or size exclusion chromatography (Tagliazucchi & Bellesia, 2015, Pastoriza & Rufián-Henares, 2014).

Melanoidins contribute to sensorial properties of food such as colour, texture, and aroma, and hence they are crucial in the consumer acceptability of the foodstuffs (Starowicz & Zieliński, 2019). Furthermore, they exert different biological activities like antioxidant, prebiotic, antihypertensive, genoprotective and anti-inflammatory properties (Del Pino-Garcia et al., 2012; Diaz-Morales, Cavia-Saiz, Salazar, Rivero-Perez, & Muniz, 2020; Echavarría, Pagán & Ibarz, 2012; Mesías & Delgado-Andrade, 2017; Nooshkam, Varidi & Verma, 2020). The antioxidant profile and colour of melanoidins have been widely studied as the reviews published by Langner & Rzeski (2014) and Nooshkam, Varidi & Verma (2020) noted. However, the antimicrobial effect of food melanoidins has been relatively less studied (Einarsson, Snygg & Eriksson, 1983; Hauser, Muller, Sauer, Augner & Pischetsrieder, 2014; Rufian-Henares & de la Cueva, 2009; Rufian-Henares & Morales 2008b; Tauer, Elss, Frischmann, Tellez & Pischetsrieder, 2004). The cited works were mainly focused on melanoidins from model systems or some specific food as coffee or beer, with scarce studies showing data about melanoidins of bakery products (Rufian-Henares & Morales, 2008a). Similarly, although previous papers noted the role of melanoidins in food aroma (Paravisini & Guichard, 2016), no available information about the volatile profile of melanoidins isolated from any foodstuff, has been found.

Additionally, melanoidins are very stable to heat treatment, so they are ideal additives for thermal-processed products as they do not degrade or lose their biological properties at high temperatures (Nooshkam, Varidi, & Verma, 2020).

The main aim of this study was to investigate the antimicrobial and volatile properties of melanoidins isolated from different bakery products and to obtain data that make easy the evaluation of their potential as alternative natural food additives. This fact can contribute to revaluating the bakery industry by-products contributing to the sustainable food production and the circular economy. This work gives novel information about the elemental composition, volatile profile, and antimicrobial properties of melanoidins isolated from common and soft bread and biscuit.

2. Material and methods

2.1. Samples

Melanoidins isolated from crusts of Maria biscuit (BM), common and soft bread (CBM and SBM) were studied after their extraction following the methods previously described in Diaz-Morales, Cavia-Saiz, Salazar, Rivero-Perez & Muniz (2020). Briefly, after scraping the dark part of biscuit, common and soft bread, obtained crusts were ground and sieved to powders of particle size lower than 1 mm (1 mm wire mesh). Melanoidins were isolated after gluten enzymatic digestion —1:6 (w/v), sieved powder/Pronase E solution (400 U/mL in 20 mM Tris-HCl buffer, pH 8.0), 37 °C and 72 h— followed by centrifugation (15,000*g*, 10 min, 4 °C), supernatants ultrafiltration (10 kDa nominal molecular weight cut-off polyethersulfone membranes), twice diafiltration of retentates with Milli-Q water, and freeze-drying.

2.2. Methods

2.2.1. Elemental composition of melanoidins

Quantitative analysis of carbon, hydrogen, nitrogen, sulfur and oxygen was performed on a Flash*Smart*™ Elemental Analyzer, (Thermo Scientific™, Waltham, Massachusetts, USA). Analysis conditions for C/H/N/S: oven temperature = 900 °C; high oxygen conditions, 250 mL/min; carrier gas He 140 mL/min and Sn capsules. Analysis conditions for oxygen: oven temperature = 1060 °C, carrier gas He 130 mL/min, and Ag capsules. Elemental analysis was carried out by triplicate, using well-known quantities of every sample (between one and two mg, 0.001 mg precision scale, Mettler Toledo XP6, Microbalance, Columbus, Ohio, USA) and results were expressed in percentage.

2.2.2. Analysis of retained volatiles

Headspace solid-phase dynamic extraction (HS-SPDE) coupled with a gas chromatograph (HP 6890N, Agilent Technologies, Inc., USA) with a mass spectrometer (Agilent 5973) fully controlled by a CTC-CombiPAL autosampler (Bender and Hobein, Switzerland) was used to analyse the volatile composition of melanoidins. A PDMS/AC fibre (Chromtech, Germany) was used after its conditioning (heating at 200 °C for 1 h). For each determination, 250 mg of each sample were introduced in a 10 mL glass vial with a metallic cap and chloro-butyl/polytetrafluoroethylene seal (Chromacol Ltd. Herts, UK). Each vial was equilibrated for 5 min at 50 °C, then 72 stokes were performed, followed by desorption into the inject port applying an injection temperature of 250 °C. Volatile compounds were separated and analysed using a Carbowax 20 M column (60 × 0.32 mm, 0.25 μm film thickness, Quadrex Corporation, Symta, Spain), helium with 1 mL/min flow rate as the carrier, oven conditions of 35 °C initial temperature, increasing 3 °C/min until 220 °C and held for 5 min, and mass detection in electronic impact mode (70 eV). The isolated volatile components were identified by comparison of the obtained mass spectra with mass spectral data from the NIST library. These analyses were carried out by triplicate and results were shown as mean values of peak areas.

2.2.3. Microbiological analysis

To assure the microbial quality of isolated melanoidins, loads of total aerobic mesophilic microorganisms (TAM), lactic acid bacteria (LAB), *Pseudomonas* spp. (PS), enterobacteriaceae (EB) and molds and yeast (M&Y) were quantified using the methods published in de-Souza-Silva et al. (2018), Garcia-Lomillo, Gonzalez-SanJose, Del Pino-Garcia, Rivero-Perez, & Muniz-Rodriguez (2014), and Tamkute, Gil, Carballido, Pukalskiene & Venskutonis (2019). Briefly, melanoidins were diluted with peptone water. Then, the spread plate technique was performed on the conditions summarized in Table 1. Results were expresses as Log of colony forming units (CFU)/g.

The antimicrobial properties of melanoidins were tested following previously published methods (Tamkute, Gil, Carballido, Pukalskiene & Venskutonis, 2019) on selected strains of the most relevant food spoilage and pathogen microorganisms, also including some molds and yeasts (Table 2). Briefly, stock cultures (stored at −70 °C in 15% glycerol) were revivificated by streaking onto the corresponding medium for 24 or 48 h at the right temperature (Table 2). An isolated colony was picked, streaked again, and incubated in the same conditions. Finally, a pure colony was suspended in 5 mL of the suitable medium and incubated o/n (18–20 h) at the right temperature. Then, broth microdilution assays (4, 2, 1 and 0.5% serial dilutions) were carried out on microorganism concentration close to 6 Log CFU/mL, which were adjusted in the base of

Table 1
Culture media and incubation conditions applied to evaluate the microbial load of melanoidins.

Microorganism	Medium	Incubation T[a]	Incubation time
Aerobic mesophilic microorganisms	PCA**	30 °C	48–72 h
Lactic acid bacteria	MRS*	30 °C	24–48 h
Pseudomonas spp.	Pseudomonas + CFC supple.*	30 °C	24–48 h
Enterobacteriaceae	VRBG ***	37 °C	24 h
Molds and Yeast	Sabouraud*	30 °C	5–10 days

CFC, Cetrimide Fucidin Cephalosporin; MRS, de Man, Rogosa and Sharpe; PCA, Plate Count Agar; Supple., Supplemented; VRBG, Violet Red Bile Glucose Agar. * Surface plate method, ** Pour plate method, *** Pour plate + anaerobic conditions.

Table 2
Microorganism (specie, strain, and shape/Gram), culture media, and incubation conditions applied in the antimicrobial assays.

Species	Strain	Shape/Gram	Incubation conditions		
			Solid selective	Tª (°C)	t (h)
Bacteria					
Enterococcus faecalis	CECT 481	Coccus/G+	Slanetz and Bartley agar	37	48
Lactobacillus brevis	CECT 4121	Rod /G+	MRS agar	30	48
Weissella viridescens	132	Coccus/G+	MRS agar	30	24
Bacillus cereus	CECT 148	Rod /G+	MYP agar	30	24
Leuconostoc mesenteroides	66	Coccus/G+	MRS agar	30	24
Staphylococcus aureus	CECT 976	Coccus/G+	Baird-Parker agar + egg yolk + tellurite	37	24
Listeria monocytogenes	CECT 934	Rod /G+	OCLA + differential and brilliance Listeria differential supple.	37	48
Clostridium perfringens (anaerobe)	CECT 376	Rod /G+	TSN agar	37	24–48
Escherichia coli	CECT 99	Rod /G-	MacConkey Agar	37	24
Salmonella spp.	UBU 132	Rod /G-	XLD agar	37	24
Campylobacter jejuni (microaerophilic)	CECT 7572	Rod /G-	mCCDA + selective supple.	42	48
Pseudomonas putida	CECT 324	Rod /G-	*Pseudomonas* agar + CFC supple.	30	24
Yeast					
Candida parapsilosis	CECT 1968		Yeast mould agar	26	24–48
Candida albicans	CECT 1394		Yeast mould agar	26	24–48
Saccharomyces cerevisiae	CECT 13,074		Yeast mould agar	26	24–48
Molds					
Mucor mucedo	CECT 2653		Potato dextrose agar	26	24
Penicillium charlesii	CECT 20,937		Potato dextrose agar	26	24
Trichoderma asperellum	CECT 20,725		Potato dextrose agar	26	24
Lichtheimia corymbifera	CECT 2218		Potato dextrose agar	26	24
Fusarium avenacum	CECT 2218		Potato dextrose agar	26	24
Aspergillus niger	CECT 2907		Potato dextrose agar	26	24
Lichtheimia corymbifera	CECT 20,164		Potato dextrose agar	30–32	24
Aspergillus flavus	CECT 20,802		Malt extract agar	26	24
Cladosporium cladosporioides	CECT 20,805		Malt extract agar	26	24
Trichoderma viride	CECT 2939		Malt extract agar	26	24
Microsporurm gypseum	CECT 2908		Saboraud chloramphenicol agar	26	24

CECT, Colección Española Cultivos tipo (Spanish Type culture collection); CFC, Cetrimide Fucidin Cephalosporin; UBU: University of Burgos; mCCDA, modified Charcoal Cefoperazone Deoxycholate Agar; MRS, de Man, Rogosa and Sharpe; MYP, Mannitol egg Yolk Polymyxin; OCLA, Oxoid Chromogenic *Listeria* Agar; TSN, Tryptone Sulphite Neomycin; XLD, Xylose Lysine Deoxycholate.

the optical density to 610 nm (Garcia-Lomillo, Gonzalez-SanJose, Del Pino-Garcia, Rivero-Perez & Muniz-Rodriguez, 2017). Broth microdilution method was also applied in the case of molds (Arendrup et al., 2020; de-Souza-Silva et al., 2018). Each studied microorganism was grown, under optimal growing conditions, in the absence or presence of melanoidins (until 4%). This concentration was the lowest that showed an antimicrobial effect in previous assays, (supplementary data), carried out with different doses of melanoidins isolated from commercial common bread. The antimicrobial effect was evaluated by comparing the growth after 24 h of incubation in the control samples and the samples with melanoidins. Assays were carried out in triplicate.

2.3. Statistical analysis

Results were statistically analysed and compared by one-way analysis of variance (ANOVA), while Tukey's multiple comparison post-hoc test pointed out the statistically significant differences considering p values ranged from 0.05 to 0.001.

3. Results and discussion

3.1. Melanoidins from bread and biscuit: elemental elements

The elemental analysis of the melanoidins (mean values, n = 3) did not detect S, and showed levels of N (3.9%, 2.4% and 3.0%); C (38.9%, 40.7% and 40.1%); H (5.9%, 6.3% and 6.0%); O (42.9%, 46.9% and 42.7%), and C/N ratio values (10.0, 11.4 and 13.5) for CBM, SBM and BM, respectively. The highest value of N, found in CBM, could be explained since common bread is made with high protein flour, while soft bread and biscuit are made from flour with low levels of protein. Besides, sugars used to make biscuits and soft bread could explain the higher levels of C and O in the melanoidins isolated from these products.

Usually, on the basis that samples only contain C, N, H, and O, the oxygen content is quantified by difference from C, N, and H percentage data (O% = 100 – C%-N%-H%) (Kang, 2016; Mohsin et al., 2018). The results obtained in this study showed quantitively high oxygen levels by sample combustion method than those obtained by difference of the percentage of the other main elements, being the last values significantly higher. Differences found between the oxygen levels quantified by the two methods were 3.8 (50.7% obtained by subtraction method – 46.9% obtained by sample combustion) for SBM; 8.3 (51.0 – 42.7) for BM, and 8.4 (51.3 – 42.9) for CBM.

3.2. Retained volatile composition

Some volatile compounds were detected when the volatile profile of isolated melanoidins was evaluated. This fact is mainly due to the capacity of melanoidins to interact and retain small MRP and other volatiles (Ortega-Heras & González-Sanjosé, 2003; Paravisini & Guichard, 2016).

A total of 29 volatile compounds were identified (Table 3). Alcohols, acids, and esters were mainly formed during the fermentation steps; ketones can result from fermentation and lipid oxidation, which also renders aldehydes, furans, and pyrazines active MRP. Besides, all cited volatiles can also be present in the raw ingredients or can be formed by thermal degradation of the ingredient components (Starowicz & Zieliński, 2019).

Levels of volatiles revealed statistically significant differences among bakery products, which were mainly due to the ingredients of each product and to the different elaboration processes. It is well-known that several factors —such as the type of sugars and amino acids, temperature and time of baking, moisture, pH, water activity and enzymes used— influence the number of volatile compounds generated during baking (Pico, Bernal & Gómez, 2015). Furthermore, concerning bread, the strains of yeast and lactic acid bacteria are also relevant (Pétel, Onno, & Prost, 2017).

Table 3
Main volatile compounds identified in biscuit and bread melanoidins.

Groups	Compounds	Biscuit	Common Bread	Soft Bread
Alcohols	Isobutanol	5.94 ± 3.63	nd	nd
	1-Butanol	nd	52.8 ± 7.0	nd
	Isoamyl Alcohol	532 ± 73 a	609 ± 59 a	nd
	2,3-Butanediol	3.43 ± 0.02 a	4.57 ± 3.72 a	nd
	Benzyl Alcohol	0.760 ± 0.002 a	2.37 ± 0.46b	nd
	Phenylethyl Alcohol	87.4 ± 11.0 a	113 ± 19 a	nd
	Total	***630 ± 85 a***	***782 ± 103 a***	***nd***
Acids	Lactic Acid	212 ± 22b	554 ± 43c	3.80 ± 0.31 a
	Acetic Acid	438 ± 69 ab	686 ± 78b	150 ± 22 a
	Propanoic Acid	nd	nd	9.50 ± 1.50 a
	Butanoic Acid	23.4 ± 14.6 a	29.5 ± 1.1 a	nd
	Hexanoic Acid	nd	11.5 ± 2.0 a	6.52 ± 2.54 a
	Heptanoic Acid	nd	2.28 ± 0.25	nd
	Octanoic Acid	0.369 ± 0.020 a	15.1 ± 2.1b	nd
	Nonanoic Acid	0.999 ± 0.240 a	2.54 ± 0.10b	nd
	Isovaleric Acid	13.7 ± 2.5 a	15.2 ± 4.5 a	nd
	Total	***689 ± 108b***	***1316 ± 52b***	***170 ± 25 a***
Aldehydes and Ketones	Diacetyl	nd	75.6 ± 28.9 a	91.6 ± 11.5 a
	Acetoin	822 ± 150b	1638 ± 191c	308 ± 45 a
	Nonanal	nd	nd	0.977 ± 0.142
	Benzaldehyde	nd	1.10 ± 0.19	nd
	Acetophenone	nd	58.5 ± 2.3b	2.53 ± 0.09 a
	Total	***822 ± 150b***	***1773 ± 128c***	***403 ± 21 a***
Furans	5-methyl-2(3H)-furanone	3.58 ± 0.79 a	1.22 ± 0.18 a	nd
	2-furanmethanol	5.51 ± 1.77b	0.852 ± 0.093 a	nd
	γ-decalactone	nd	4.00 ± 0.30	nd
	Total	***9.09 ± 2.56 a***	***6.07 ± 0.21 a***	***nd***
Pyrazines	Methyl pyrazine	27.0 ± 0.2b	13.9 ± 1.6 a	nd
	2,3-dimethylpyrazine	nd	2.57 ± 0.40	nd
	Total	***27.0 ± 0.2b***	***16.5 ± 2.0 a***	***nd***
Other compounds	Limonene	14.6 ± 1.85 a	14.7 ± 2.7 a	13.2 ± 2.6 a
	m-cymene	nd	nd	320 ± 39
	3-methylthio-1-propanol	2.09 ± 0.13 a	8.03 ± 0.91b	nd
	2-acetyl-pyrrole	nd	0.698 ± 0.109	nd
Total volatiles		***2194 ± 424b***	***3911 ± 108c***	***906 ± 26 a***

Values: mean values of peak area × 10^{-6} ± standard deviation. Different letters indicate significant differences ($p < 0.05$) (n = 3). nd = no detected

CBM showed the highest number of volatile compounds and at the highest amounts. These results indicate that CBM has more aromatic potential than SBM and BM, thus suggesting a higher capacity to enrich volatile profile and, probably, to enhance odour and aroma of food supplemented with them. This hypothesis is reinforced by the fact that two pyrazines were identified in CBM, but only methyl pyrazine was detected in BM and none in SBM. Similarly, furans were only detected in CBM and BM. Pyrazines are directly correlated with roasty and nutty olfactory notes, all of them with a positive effect on food aroma (García-Lomillo, González-SanJosé, Del Pino-García, Ortega-Heras & Muñiz-Rodríguez, 2016).

A notable fact was that neither furfural nor 5-hydroxy-methyl-furfural were found in the melanoidins studied. This is a positive feature since these two MR compounds have a toxic effect on human health. In this sense, furfural may lead to hepatoxicity (Mesias, Delgado-Andrade & Morales, 2019) and 5-hydroxy-methyl-furfural can be biotransformed into the toxic and mutagenic metabolite sulphoxy-methyl-furfural (Pastoriza de la Cueva et al., 2017). That furfural and 5-hydroxy-methyl-furfural were not detected agrees with the results of a previous work which showed the melanoidins used in this study did not have cytotoxicity (Diaz-Morales, Cavia-Saiz, Salazar, Rivero-Perez, & Muniz, 2020). On the contrary, furfuryl alcohol was found. This compound gives pleasant aromatic notes (Pico, Antolín, Román, Bernal & Gómez, 2019), and it is obtained from the reduction of furfural (Spillman, Pollnitz, Liacopoulos, Pardon & Sefton, 1998) and, at low pH values, polymerises to aliphatic polymers that give a brown colour to bread (Okaru & Lachenmeier, 2017).

3.3. Microbial quality and antimicrobial activities of melanoidins from bread and biscuit

The microbial load of isolated melanoidins was adequate for its use in the food industry (Commission Regulation (CE), 2005). Neither EB nor PS was present in the studied melanoidins. However, in BM load of 3.61 ± 0.33 Log CFU/g of TAM were detected; while CBM contained TAM (4.39 ± 0.90) and LAB (3.38 ± 0.64), and SBM had TAM (3.50 ± 0.74), LAB (3.49 ± 1.07), and M&Y (3.02 ± 0.21).

The three melanoidins showed some significant antimicrobial properties at 4% (Table 4). In general, CBM showed the highest antimicrobial activity, showing bactericide effect (a total reduction of initial count) against *Clostridium perfringens,* and significant reduction of the initial count for the other bacterial strains studied, except for *E. faecalis* and *L. brevis,* that showed bacteriostatic effect (no increment of bacterial count). On the contrary, SBM exhibited the lowest antimicrobial activities, with a significant reduction of the initial count only against

Table 4
Antimicrobial effect of melanoidins against different bacteria.

Bacteria	Biscuit melanoidins		Soft bread melanoidins		Common bread melanoidins	
	4%	2%	4%	2%	4%	2%
Enterococcus faecalis	$**		$*		=	ND
Lactobacillus brevis	$**		ND		=	$*
Weissella viridescens	$*		ND		ϕ*	ND
Bacillus cereus	ND		ND		ϕ*	$*
Leuconostoc mesenteroides	$**		$**		ϕ*	$**
Staphylococcus aureus	ND		$*		ϕ*	ND
Listeria monocytogenes	$***		=		ϕ**	$*
Clostridium perfringens	ϕ**	=	=		ϕ***	$**
Escherichia coli	$**		ND		ϕ**	$*
Salmonella spp.	ND		$*		ϕ**	ND
Campylobacter jejuni	ϕ**	ϕ**	ϕ**	ϕ**	ϕ**	ϕ**
Pseudomonas putida	ϕ**	ND	=		ϕ**	ϕ*

ϕ: reduction of bacterial count respect the initial count; ϕ ***: bactericide effect (total reduction of initial count); ϕ** (final count more than 3 log lower than initial count; ϕ* (final count from 1 to 3 log lower than initial count); =: bacteriostatic effect (initial and final count statistically no different). ND: antibacterial effect no detected (bacterial growth occurs similarly to control). $: Growth reduction with significant differences respect control without melanoidins; $*** reduction of more than 3 log. $** reduction from 1 to 3 log; $*: reduction of 1 log. All reductions were statistically significant ($p < 0.05$).

C. jejuni and bacteriostatic effect against *L. monocytogenes, Cl. perfringens* and *Ps. putida.* Furthermore, SBM lead to a significant inhibition of the growth (reduction with respect to control) of *E. faecalis, Lc. mesenteroides, S. aureus, Salmonella* spp. Antimicrobial activity of BM, although higher than that of SBM, was lower than CBM, showing a significant reduction of the initial count against *Cl. Perfringens, C. jejuni* and *Ps. putida,* and, in general, significant inhibition of the growth of the other studied bacteria.

In the cases in which the concentration of 4% produced reduction of the initial count, the effect of lower concentration (2%) is also showed (Table 4). In general, the lower the concentration of melanoidins, the lower their antimicrobial effect. A 2% concentration of the three melanoidins showed a significant reduction of the initial count against *C. jejuni,* but not against other bacteria. Only 2% of CBM reduced the growth (respect to control) of the other studied bacteria but it was not able to reduce the initial count (Table 4). Furthermore, 1% CBM reduced the growth of *L. monocytogenes* and *W. viridescens*, and even 0.5% CBM decreased the growth of the last bacteria.

Globally, obtained results were better against Gram – bacteria. This fact is contrary to those observed by Einarsson, Snygg & Eriksson (1983), Hauser, Muller, Sauer, Augner & Pischetsrieder (2014), Rufian-Henares and de la Cueva (2009) and Rufian-Henares and Morales (2008a), who indicated a higher antimicrobial effect of melanoidins isolated from coffee and biscuit against Gram + bacteria, with MIC (minimum inhibitory concentration) of 4 mg/mL for *B. cereus* and *St. aureus;* 4–4.5 mg/mL for *Proteus mirabilis, Ps aeruginosa* and *S. typhimurium,* and >5 mg/mL for *E. coli* and *Ps. aeruginosa.* This contradiction could be due to the different structures of the melanoidins used in each study.

The studied melanoidins (at 4%) show no effect against three yeasts assayed. Observed results contrast to those indicated by Tauer, Elss, Frischmann, Tellez, and Pischetsrieder (2004), who observed a negative effect of products derived from thermally processed mixtures of carbohydrate/amino-acid on the fermentation by *S. cerevisiae.*

The antifungal effect of the melanoidins was variable among melanoidins and molds. Thus, CBM (4%) showed antifungal activity against *Penicillium charlessii, Fusarium avenaceum* and *Microsporum gypseum,* whiles SBM (4%) showed antifungal effect only on the two first cited molds, and BM (4%) show no antifungal activity. The antifungal effect was not observed to lower concentrations (2%). Low information about the antifungal properties of melanoidins or other Maillard reaction compounds has been found. Kuwabara, Simizu, and Yajima (1972) noted antifungal effects of Maillard derived products (glutamic-acid-xylose) against P*enicillium glaucum, Aspergillus oryzae,* and *Rhizopus nigricans.*

To summarize, data indicate that the antimicrobial properties of melanoidins are variable. Both, microorganism species and melanoidin type, are factors of variability. Obtained results showed higher antimicrobial activity of CBM than BM and SBM. This fact could be due to differences in melanoidin structure. CBM had the lowest C/N ratio and SBM the highest, which could influence their capacity to chelate metals and produce cell membrane disruption which is the mechanism proposed to explain the antimicrobial properties of melanoidins (Rufian-Henares & de la Cueva, 2009; Rufian-Henares & Morales, 2008a).

4. Conclusions

Melanoidins isolated from different bakery products have interesting technological properties that can be useful to the food industry. Results from "in vitro" antimicrobial assays indicate that bakery melanoidins, mainly those isolated from common bread crusts, have interesting antimicrobial properties to the food industry due to their capacity to improve the shelf-life of food (antimicrobial effect on spoilage microbiota) but also to improve food safety (antimicrobial effect on pathogens). On the other hand, melanoidins could modify the volatile profile of the foods where they are added, increasing the number and quantity of volatile compounds, and then could modify, directly or indirectly (synergism), the aroma of foodstuffs.

Results showed in this paper contribute to the knowledge about melanoidins properties, giving novel information about melanoidins isolated from bakery by-products. The new data provided reinforce the hypothesis that bakery melanoidins could be used as natural food ingredients or additives contributing to sustainable food production and the circular economy.

CRediT authorship contribution statement

Noelia Diaz-Morales: Methodology, Formal analysis, Investigation, Writing – review & editing. **Miriam Ortega-Heras:** Methodology, Investigation, Validation, Writing – review & editing. **Ana M. Diez-Maté:** Methodology, Investigation, Validation, Writing – review & editing. **Maria L. Gonzalez-SanJose:** Conceptualization, Methodology, Supervision, Resources, Writing – original draft. **Pilar Muñiz:** Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.131648.

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