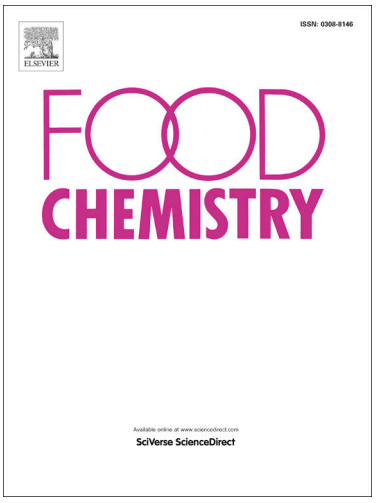
Journal Pre-proofs



Cytotoxicity study of bakery product melanoidins on intestinal and endothelial cell lines

Noelia Diaz-Morales, Mónica Cavia-Saiz, Gonzalo Salazar, M Dolores Rivero-Pérez, Pilar Muñiz

PII: S0308-8146(20)32267-6
DOI: https://doi.org/10.1016/j.foodchem.2020.128405
Reference: FOCH 128405

To appear in: *Food Chemistry*

Received Date: 21 May 2020
Revised Date: 14 September 2020
Accepted Date: 12 October 2020

Please cite this article as: Diaz-Morales, N., Cavia-Saiz, M., Salazar, G., Dolores Rivero-Pérez, M., Muñiz, P., Cytotoxicity study of bakery product melanoidins on intestinal and endothelial cell lines, *Food Chemistry* (2020), doi: https://doi.org/10.1016/j.foodchem.2020.128405

Cytotoxicity study of bakery product melanoidins on intestinal and endothelial cell lines.

Noelia DIAZ-MORALES[a], Mónica CAVIA-SAIZ[a], Gonzalo SALAZAR[a], M Dolores RIVERO-PÉREZ[a], Pilar MUÑIZ[a]*

[a] Department of Biotechnology and Food Science, Faculty of Sciences, University of Burgos, Plaza Misael Bañuelos, 09001, Burgos, Spain.

***Corresponding author**: Dr. Pilar Muñiz Rodríguez, Plaza Misael Bañuelos, Facultad de Ciencias, Departamento de Biotecnología y Ciencia de los Alimentos, 09001, Burgos, Spain.

E-mail: pmuniz@ubu.es

Phone: +34-947258800 Ext. 8210

Fax: +34-947258831

Email addresses: Noelia Diaz-Morales (ndiaz@ubu.es), Mónica Cavia-Saiz (monicacs@ubu.es), María D. Rivero-Pérez (drivero@ubu.es), Gonzalo Salazar Mardones (gsalazar@ubu.es), Pilar Muñiz (pmuniz@ubu.es).

ORCID iDs:
Noelia Diaz-Morales https://orcid.org/0000-0003-1657-2700
Mónica Cavia-Saiz https://orcid.org/0000-0001-5132-381X
María D. Rivero-Pérez https://orcid.org/0000-0003-0907-4009
Gonzalo Salazar https://orcid.org/0000-0003-2172-4270
Pilar Muñiz https://orcid.org/0000-0002-9306-0082

Abstract

Melanoidins contribute to organoleptic properties of processed foods and exert benefits in health. The aim of this study was to isolate and characterize melanoidins from baked products (common bread, soft bread and biscuits), evaluate their cytotoxicity and determine their suitability as functional additives. Extraction yield, spectrophotometric characteristics, colorimetric properties, antioxidant capacity, and cytotoxicity of melanoidins were assessed. Among the studied products, soft bread had the highest extraction throughput. Melanoidins from biscuit showed the highest antioxidant capacity, closely followed by those of soft bread. Melanoidins did not exert cytotoxic effects on Caco-2 and HUVEC cells (viability was >80%). Nevertheless, incubation of HUVEC cells with melanoidins from common bread and biscuit slightly decreased viability, whereas gastrointestinal digestion of such melanoidins softened the decrease in cell viability. This study point to soft bread as a safe and efficient source of melanoidins, that could be potentially used in the future as functional ingredient.

Keywords

Melanoidins, bakery products, soft bread, biscuits, common bread, antioxidant capacity, bioaccesibility, cytotoxicity.

1. Introduction

Melanoidins are products of the Maillard reaction (MR) formed during the last stages as a result of chemical reactions between the carbonyl group of reducing sugars and the amino group of amino acids or peptides. MR is a common non-enzymatic browning transformation which takes place in food during thermal treatment, cooking or preservation, and contributes to the organoleptic characteristics, including aroma or colour, in processed food (Mesías & Delgado-Andrade, 2017). Therefore, melanoidins are brown coloured, heterogeneous, polymeric high molecular weight compounds present in a wide variety of food, such as bakery products and coffee (Alves, Xavier, Limoeiro & Perrone, 2020, Fogliano & Morales, 2011, Gonzalez-Mateo, Gonzalez-Sanjose & Muñiz, 2009), cocoa beans (Oracz & Zyzelewicz, 2019), sweet wine (Goulas, Nicolaou, Botsaris & Barbouti, 2018, Ortega-Heras & González-Sanjosé, 2009), etc, which make them a part of the general population daily diet. In this sense, it has been estimated that around 10-12 grams of melanoidins from all the possible alimentary sources are ingested everyday by general population (Alves, Xavier, Limoeiro & Perrone, 2020, Fogliano & Morales, 2011, Pastoriza & Rufián-Henares, 2014, Perez-Burillo, Rajakaruna, Pastoriza, Paliy & Angel Rufian-Henares, 2020). In Spain, melanoidins are mainly ingested from bakery products, and the intake of melanoidins isolated from bread ranges between 1.8 to 15 g per day and from biscuits is around 3.2-8.5 g/day (Fogliano and Morales 2011).

A growing body of evidence has proved in the last years that melanoidins play a crucial role in different biological activities, and have diverse functional properties that can exert potential benefits in human health. Thus, it has been demonstrated that, besides to contribute to technological qualities as texture, flavour and colour, melanoidins have antioxidant, prebiotic, antimicrobial, antihypertensive and anti-inflammatory properties (Mesías & Delgado-Andrade, 2017, Nooshkam, Varidi & Verma, 2020). Antioxidant activity relies on the ability of melanoidins to chelate metal ions (e.g. iron) leading to the formation of inactive complexes, or their capacity to reduce and stabilize superoxide, hydroxyl or peroxyl radicals, thus improving

shelf-life of the food (Nooshkam, Varidi & Verma, 2020, Passos et al., 2017). According to the antioxidant activity of melanoidins, *in vitro* assays on human HepG2 cells have demonstrated that incubation with biscuit melanoidins enhanced antioxidant defences and reduced free radical activity, thus protecting cells against oxidative challenge (Martin et al., 2009). Studies about the melanoidins contribution on the overall antioxidant capacity estimated that melanoidins contribute 20.2 % to the daily intake of antioxidant capacity in the Spanish diet, being coffee and biscuits two of the main contributors (Pastoriza & Rufián-Henares, 2014).

Given the importance of these characteristics, melanoidins are being studied as good candidates to be part of functional food components, which might be used to supplement processed food and potentially contribute to improving their taste, shelf-life and healthiness (Mesías & Delgado-Andrade, 2017). Furthermore, there are more and more requests from food industry to discover and characterize new functional ingredients which, when incorporated to foodstuff, potentially improve the final product properties. In order to respond such demand, the hypothesis of this study is that melanoidins isolated from by-products of bakery foods, such as the crust of soft bread or discarded common bread or biscuits, might present different antioxidant characteristics and might affect cell viability (themselves or their bioaccessible fractions), thus making them more (or less) appropriate as functional ingredients. The potential use of by-products derived from bakery industry to isolate functional ingredients would increase the added value of such waste products. The aim of this work was to isolate and characterize melanoidins from the three different bakery products or by-products, compare their properties, and evaluate the cytotoxicity of the melanoidins themselves and their bioaccesible fractions. For this purpose, the current study have obtained melanoidins from crusts of common bread, soft bread and biscuit and have assessed extraction yield, antioxidant capacity, spectrophotometric and colorimetric characteristics of the isolated melanoidins, and cytotoxicity (on Caco-2 and HUVEC cells) of melanoidins and of their bioaccessibles fractions after gastro-intestinal digestion.

Material & methods

1.1.Reagents

Pronase E (EC 3.4.24.4, 4,000,000 U/g, #107433); 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, >98%, #A1888); Potassium persulfate ($K_2S_2O_8$, >99%, #P5592); 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX, 97%, #238813); 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%, #D211400); α-Amylase from porcine pancreas (EC 3.2.1.1; 22 U/mg, #A3176); Pepsin from porcine gastric mucosa (EC 3.4.23.1; 474 U/mg, #P7000); Pancreatin from porcine pancreas (EC 232-468-9; 4U/mg, #P1750); Bile salts (#B8756); Sodium chloride (NaCl, 99.5%, #106404); Minimum essential medium Eagle (MEM, #M2279); Dulbecco's Modified Eagle's Medium (DMEM, #D5546); Fetal Bovine Serum (#F0804); Penicillin-Streptomycin solution (10,000 units penicillin and 10 mg streptomycin/ml, #P4333); L-Glutamine solution (200 mM, #G7513); MEM non-essential amino acid solution (100×, #M7145); Sodium pyruvate solution (100 mM, #S8636); Trypsin-EDTA solution (2.5 g/l porcine trypsin and 0.2 g/l EDTA · 4Na, #T4049); and Thiazolyl blue tetrazolium bromide (MTT,98% #M2128) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl, 37%, #CHAC-OIA) was obtained from Labbox Labware SL (Barcelona, Spain). Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, >97%, #141358); Sodium bicarbonate ($NaHCO_3$, >99.7%, #131638); and Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$, >98%, #141965) were purchased from Panreac Química SLU (Barcelona, Spain). Monopotassium phosphate (KH_2PO_4, >98%, #26922.295); and Sodium acetate ($C_2H_3NaO_2$, >99%, #27653.260) were obtained from VWR Chemicals (Radnor, PA, USA). Iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, >99%, #092120) was purchased from Probus S.A. (Badalona, Spain). Tris(hydroxymethyl)aminomethane hydrochloride ($C_4H_{11}NO_3 \cdot HCl$, TRIS, 99.8%, #10424521); Trichloroacetic acid ($C_2HCl_3O_2$, >99.5%, #1076496); Acetic acid (CH_3COOH, >99.6%, #1046072); Methanol (CH_3OH, >95%, #10506279);

Calcium chloride ($CaCl_2$, >95%, #11488093); Potassium chloride (KCl, >99%, #196770010); Sodium phosphate dibasic (Na_2HPO_4, >95%, #10214020); Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, >99%, #10386743); 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ, >99%, #10082120); Ammonium carbonate ($(NH_4)_2CO_3$, >95%, #10511272); and Dimethyl sulfoxide (DMSO, >99%, #1008011) were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

1.2. Bakery samples

Crust of three types of bakery products (soft bread, biscuits and common bread) were used in this study. Commercial soft bread was obtained from Cerealto-Siro Company as by-products, biscuits (type Maria) from a local supermarket, and common bread was prepared in the pilot plant of the Food Technology Department of University of Burgos (Spain).

Common bread was prepared from 1 kg of wheat flour, 20 g of salt, 28 g of fresh yeast, and ~500 ml of water. All the ingredients were first mixed in a kitchen mixer (KitchenAid, KSM90, MI, USA) and then manually kneaded. Dough was left to develop for 40 min at room temperature, and then was divided into 50 g portions which were rounded and placed in a fermentation cabinet (Salva Industrial SA, Guipuzcoa, Spain) for 30 min at 28 ºC and high humidity. After the first fermentation, dough was punched down, folded to the final shape and placed again in the fermentation cabinet for 90 min. The proofed dough was baked in an electrical convection oven (Berto's, Padova, Italy) at 220 ± 5 ºC for 20 min to obtain common bread rolls.

Separation of the crust from the attached crumb in the common bread rolls and the soft bread by-products was made by scrapping their surface with a kitchen knife thus obtaining the brown coloured part (crust) and discarding the white crumb. For biscuits, a scalpel was used to scratch their surface and obtain only the brown superficial part. For all the three products, the obtained samples were ground in a coffee mill (Taurus Minimoka GR20, Taurus Group, Oliana,

Spain) and sieved to a particle size < 1 mm with a 1 mm wire mesh sieve (CISA, Barcelona, Spain).

1.3.Isolation of melanoidins

Melanoidins from the three-bakery products were extracted by *in vitro* digestion with Pronase E (protease that allows gluten solubilisation, thus allowing MRPs release from the gluten network) according to the methods described by Gonzalez-Mateo, Gonzalez-Sanjose & Muñiz (2009) and Roncero-Ramos *et al*. (2013) with slight modifications. Briefly, 125 g of crust powder were enzymatically hydrolysed with 750 ml of a 400 U/ml solution of pronase E in 20 mM Tris-HCl buffer (pH 8.0), for 72 h at 37 ºC with continuous agitation in digestion jars of a Daisy^{II} incubator (ANKOM Technology Corporation, NY, USA). *In vitro* digestion reactions were stopped by mixing the samples with 15 ml of 40 % trichloroacetic acid solution (w/v) and lowering their temperature during subsequent centrifugation (15000 g at 4 ºC for 10 min). 500 ml of soluble fraction were subjected to ultrafiltration in a Millipore stirred cell (Model 8050, Millipore corporation, MA, USA) equipped with a 10 kDa nominal molecular mass cutoff polyethersulfone membrane (Trisep Flat Sheet Membrane UF10, Sterlitech Corporation, WA, USA) and the retentate, containing melanoidins, was diafiltrated twice with milli-Q water. Melanoidins (the retained fraction) were freeze-dried (FreeZone 12 L Console Freeze Dry System with drying chamber, Labconco, MO, USA), and weight to determine the extraction yield per 100 g of crust. Melanoidins extraction of the three bakery products was performed in triplicate.

1.4.Colorimetric measures

For bakery products, colour was evaluated directly on the surface of each sample, whereas powder melanoidin extracts were placed on a white paper covering an approximate area of 3 cm^2. Colour was determined with a colorimeter CM-2600d (Konica Minolta, Tokyo, Japan), under conditions established by the “Commission Internationale de l’Eclairage”, CIE, (D65

standard illuminant and 10° observation angle), with specular component excluded (SCE). Colour coordinates were defined on the CIELAB colour space to assess the L* (lightness), a* (red/green hue) and b* (yellow/blue hue) coordinates for each sample (International Commission on Illumination, 2004). At least three measures were taken from each individual sample.

1.5.Absorbance of melanoidins

The melanoidin content in the extracts was evaluated spectrophotometrically following the procedure described by Ortega-Heras & González-Sanjosé (2009). Samples of the extracted melanoidins were dissolved in milli-Q water at a concentration of 10 mg/ml to have an initial solution that was then 1:3 diluted to obtain the working solution of samples. Absorbance at 345, 360 and 420 nm (browning indexes) were measured in the working samples using a spectrophotometer (U-2000 Hitachi, Ltd., Hubbardston, MA, USA) and 1 cm-path length cuvettes. Measurements were performed in triplicate.

1.6.Antioxidant capacity

Three different methods were used to assess antioxidant activity of the melanoidins extracted from soft bread, common bread and biscuits. Assays to estimate the $ABTS^{\bullet+}$ radical scavenging activity, the capacity to reduce the $DPPH^{\bullet+}$ radical, and the ferric reducing antioxidant power (FRAP) were performed following the procedure described by Del Pino-Garcia *et al.* (2015) with modifications. Five measures were made from each sample.

1.6.1. ABTS assay

$ABTS^{\bullet+}$ cation was produced by 1:1 mixing of 7 mM ABTS solution and 2.45 mM potassium persulfate. The mix was allowed to react for at least 16 h in darkness at room temperature, and then water diluted to an absorbance of ~0.7 at 734 nm. Sample reactions were made by incubating 5 mg of the powdered melanoidins with 6 ml of diluted ABTS solution in the dark

with continuous shaking for 30 min. After centrifugation at 10500 g for 3 min, supernatant absorbance at 734 nm was measured spectrophotometrically. Calibration curve was performed with different amounts of Trolox, and results were expressed as µg of Trolox equivalents (TE) per mg of melanoidins.

1.6.2. DPPH assay

A 0.1 mM $DPPH^{\bullet+}$ solution was prepared and methanol diluted to achieve a final absorbance at 517 nm of ~0.7. Then, 5 mg of the freeze-dried melanoidins were allowed to react with 6 ml of DPPH working solution for 30 min at room temperature under stirring and darkness. Centrifugation at 10500 g for 3 min was performed, and absorbance reading of the supernatant was taken for each sample and subtracted from the $DPPH^{\bullet+}$ radical absorbance. Trolox was used again to obtain the calibration curve, and results were expressed as µg of TE / mg of melanoidins.

1.6.3. FRAP assay

FRAP reactive was freshly obtained by mixing 0.3 M sodium acetate buffer (pH 3.6), 20 mM $FeCl_3 \cdot 6\text{-}H_2O$, 10 mM TPTZ, and milli-Q water in 10:1:1:1.2 (v/v) proportion. The ferric reducing ability was assessed by incubating 6 ml of FRAP reactive with 5 mg of melanoidins for 30 min in a shaking water bath at 37 ºC in the dark. Following 10500 g centrifugation for 3 min, absorbance at 593 nm was measured and results expressed as µg of $FeSO_4$ / mg of melanoidins. Linear calibration was made using known concentrations of $FeSO_4$.

1.7. *In vitro* gastrointestinal digestion

In order to mimic physiological process, melanoidins were subjected to an *in vitro* gastrointestinal digestion according to the protocol described by Minekus *et al*. (2014) with some modifications. Concisely, an initial oral phase was performed by incubating 500 mg of melanoidins in 10 ml of a simulated salivary fluid (pH 7) containing α-amylase (75 U/ml) for 2

min at 37 ºC. Then, pH was adjusted to 1.5 with 1 M HCl, and 1000 U/ml pepsin solution (dissolved in 0.2 M HCl-KCl buffer, pH 1.5) was added in order to achieve a final pepsin concentration of 500 U/ml. This simulated gastric phase was incubated for 2 h at 37 ºC in an orbital shaker (100 rpm). At that point, 1M $NaHCO_3$ solution was added to adjust pH to 7.5; and intestinal phase started after the addition of a solution of 0.1 M sodium phosphate buffer (pH 7.5) containing 255 U/ml pancreatin and 58,3 mg of bile salts to achieve a final concentration in the sample of 100 U/ml pancreatin and 10 mM bile salts. The intestinal digestion was carried out during 2 h at 37 ºC in a thermostatic orbital shaker (100 rpm). The resultant gastrointestinal digested solution was centrifuged (5300 g, 10 min), and the soluble portion (supernatant), considered as the bioaccesible fraction, was freeze dried (Labconco, MO, USA) and stored at -20 ºC until use.

1.8.Cell viability assays

1.8.1. Cell culture

Human colon adenocarcinoma Caco-2 (ATCC® HTB-37™) cells were purchased from the American Type Culture Collection (ATCC). Cells were cultured in Eagle's minimum essential medium (MEM). The culture medium was supplemented with 20 % (v/v) heat-inactivated fetal bovine serum, 1 % (v/v) non essential amino acids, 1 % (v/v) L-glutamine, 1 % (v/v) sodium pyruvate, and 1 % (v/v) penicillin/streptomycin.

The immortalised human umbilical vein endothelial cell line (HUVEC EA.hy926) was used in experiments. HUVEC cells were cultured in DMEM supplemented with 10 % heat-inactivated fetal bovine serum, 1 % L-glutamine and 1 % penicillin/streptomycin. Cells were cultured in a 90 % humidity atmosphere with 5 % CO_2 at 37 ºC, the culture medium was changed every 2 or 3 days and subculture was performed at 70 – 80 % confluence for Caco-2 and 80 – 90 % for HUVEC cells.

1.8.2. MTT assays

The potential cytotoxic effect of the melanoidins and their bioaccesible fractions was studied in Caco-2 and HUVEC cells as representative of the human colon and endothelium respectively. Cells were seeded in 96-well microplates at a density of 1 x 10^4 cells per well, and pre-incubated for 24 h to allow them to attach and proliferate. The cells were then exposed to different concentrations of melanoidins themselves and their respective digested fractions (25, 50, 100 and 200 µg/ml) in complete culture medium for 24 h. Non-treated control was incubated with just medium. After treatment, MTT (PBS dissolved) was added to each well to a final concentration of 0.5 mg/ml, and incubated for 120 min (HUVEC) or 150 min (Caco-2) at 37 ºC. The medium was then aspirated and the formazan crystals were dissolved in 100 µl DMSO / well. Absorbance at 570 nm was measured in a PowerWave XS2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). Relative cell viability was expressed as percentage of the non-treated cells (control). The % of viable cells in untreated control was defined as 100 % (n = 3).

1.9.Statistical analysis

Results were statistically analysed using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA). Data were expressed as mean ± standard deviation, and compared by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. When comparisons were carried out against the control (this is the case of cell viability assays), Dunnet's multiple comparison test was conducted. Statistically significant differences were considered when $p < 0.05$.

2. Results and discussion

In the current study the physic-chemical characteristics of melanoidins extracted from the crust of three bakery products (soft white bread, common bread and biscuits) were evaluated, as well as the impact of such melanoidins and their digested fractions on the viability of two different cell lines.

The melanoidins intake in human diet is important, with an average consumption of 10 g melanoidins per day (Alves, Xavier, Limoeiro & Perrone, 2020, Fogliano & Morales, 2011, Pastoriza and Rufián-Henares, 2014). Bakery products are important components of the Mediterranean diet, placed at the base of the food pyramid, and constitute one of the main sources of dietary melanoidins (Fogliano & Morales, 2011). With this regard, bread (mainly common bread) contributes to a healthy Mediterranean diet, whereas consumption of biscuits and soft bread (due to their high amount of sugars and hidden fat) should be occasional.

2.1. CIELAB colour space analysis of the food matrices crust.

During the baking process, gluten proteins react with coloured MR products resulting in the formation of melanoidins which, when the skeleton is mainly conformed by proteinaceous material, are also referred to as melanoproteins (Lindenmeier, Faist & Hofmann, 2002). This process occurs mainly in the crust, is highly dependent on the baking temperature and time, and is crucial in the acceptance of the final product by consumers, as it contributes to the characteristic browning of the surface (Purlis, 2010). CIELAB colour space system has been widely used in food science as standard for colour determination (Yam & Papadakis, 2004). Several works have highlighted strong positive correlations between the trend to darker colours and the amount of melanoidins in muffins (Gonzalez-Mateo, Gonzalez-Sanjose & Muñiz, 2009), honey (Brudzynski & Miotto, 2011a) and coffee beans (Borrelli, Visconti, Mennella, Anese & Fogliano, 2002). For these reasons, before extracting the melanoidins, the crust colour on the surface of the three bakery products was assessed with the CIELAB colour space system to characterize the initial products (Figure 1). Significant differences in the

lightness (L*) of the three matrices were observed. Biscuits exhibited the higher L* value and soft bread the lowest, thus indicating that soft bread displayed darker crust than common bread, and that biscuit crust was the lightest (Figure 1A). As expected, the three foods exhibited a trend towards yellowish and reddish colours in their crust, as indicated by the a* and b* positive values (Figure 1B and 1C), which is suggestive of the typical golden tonalities found on bakery food. Redness levels were higher in soft bread crust when compared to biscuits which, in turn, showed more increased values than common bread (Figure 1B). Differences in yellow tonality, although not as pronounced, were found between common bread and the other two products, where soft bread and biscuits had higher b* values than common bread (Figure 1C). It is noteworthy that factors such as amino acid and sugar composition, that are critical ingredients in bakery products, could contribute to crust browning (Shen, Chen, Li & Tebben, 2019).

2.2.Melanoidins extraction yield

Amount of melanoidins obtained per 100 g of product crust is represented in Figure 2. Higher quantities of melanoidin extracts were isolated from soft-bread-crust (17.93 ± 4.83 g of melanoidins for each 100 g of digested crust) than from common–bread-crust (9.18 ± 1.32 %) and biscuit-crust (9.73 ± 3.85 %). These results agree with the colour characteristics, where the soft-bread-crust was the darkest product (it was at the lowest L* point) and with high red and yellow tonalities, which confirms the importance of melanoidin content on the final colour of the surface. Limited throughputs of common bread and biscuit melanoidins might be related to the increased levels of lightness found in the crust of these products when compared to soft bread.

The melanoidin yield for soft bread is in agreement with data published by Fogliano and Morales (2011), which reported an amount of 18 g of melanoidins per 100 g of crust. Their study showed a slightly higher yield in common bread and biscuits (14 and 12 % respectively),

probably due to differences in dough composition, baking conditions and/or melanoidin extraction methodology. However, it is clear that in both cases soft bread had higher amount of melanoidins on its surface than common bread and biscuits (Fogliano & Morales, 2011).

2.3.Melanoidins absorbance values and colour parameters.

The formation of melanoidins was evaluated spectrophotometrically by measuring absorbance at 345 nm (Ortega-Heras & González-Sanjosé, 2009), and the presence of intermediate compounds formed during the sequential steps of the MR (which can be referred as pre-melanoidins) were quantified by measuring absorbance at 360 nm (Kang, 2016). Final compounds of the MR with high molecular weight usually present chromophore groups with a characteristic maximum absorption peak at 420 nm, such absorbance can be considered as the general browning index of MR products samples (Cho, Bae, Cho & Moon, 2017, Morales & Jiménez-Pérez, 2004).

The absorbance values obtained at 345 nm were more elevated than those at 360 nm and 420 nm for each bakery product (Figure 3A-C), indicating a high abundance of melanoidins being markedly greater in biscuit crust extracts than in those of soft and common bread (Figure 3A). The same pattern was found in pre-melanoidin content (Figure 3B) and brown final-stage products of the MR (Figure 3C), suggesting that the extracts from biscuits are the most enriched with MR products, including melanoidins; whereas common bread showed the extracts with reduced amounts of MR products.

Lightness (L*), redness (a*) and yellowness (b*) parameters were also assessed on melanoidins extracts (Figure 3D-F). The darkest tonalities were observed in melanoidins from biscuits (Figure 3D), which was accompanied by higher red tonalities when compared to soft- and common-bread melanoidins (Figure 3E) and according with the results of spectrophometric colour. Yellow pigments were found in the three melanoidin extracts, being slightly more intense in soft bread than in common bread melanoidins, whereas biscuit melanoidins showed

intermediate values of yellowness (Figure 3F). These results indicate that melanoidins are the main contributors to colour intensity within the extracts, even though the final crust colour of the product itself (bread or biscuits) in the present study must have been also influenced by other conditions not studied here, such as dough ingredients, baking temperature or time. In this sense, Brudzynsky and Miotto (2011a) observed that, despite a remarkable darkening of honey after heat-treatment, a part of brown coloured melanoidins precipitated and were discarded in the centrifugation step preliminary of absorbance measurement. In addition, in biscuits, melanoidins are uniformly distributed across the crust and inside them, whereas melanoidins of bread are mainly found in the crust (Fogliano & Morales, 2011). Consequently, the limitation in melanoidin content of soft and common bread extracts might be related to the inevitable amount of attached crumb (poorly enriched in melanoidins) that remains with the crust during the separation process.

2.4. Melanoidins antioxidant capacity

It is well known the beneficial antioxidant effect of brown MR products or melanoidin fractions isolated from a wide variety of foods such as bread (Celik, Rubio, Andersen & Gokmen, 2018), biscuits (Pastoriza & Rufián-Henares, 2014, Patrignani, Rinaldi, Rufian-Henares & Lupano, 2019), muffins (Gonzalez-Mateo, Gonzalez-Sanjose & Muñiz, 2009), coffee (Celik, Rubio, Andersen & Gokmen, 2018, Iriondo-DeHond, Ramírez, Escobar & Castillo, 2019), etc. In fact, melanoidins are considered to play a crucial role in preventing oxidative damage due to their capacity to chelate metals and their radical-scavenging activity (Mesías & Delgado-Andrade, 2017, Wang, Qian & Yao, 2011). Due to this multifunctional nature, antioxidant activity of melanoidins should be tested by several methods. For this reason, in the current study, the free radical scavenging ability was measured by the ABTS and DPPH protocols, while the iron reducing capacity was assessed by the FRAP method. Antioxidant capacity of the isolated melanoidins is reported in Figure 4. The higher radical scavenging ability (ABTS and DPPH, Figure 4A and 4B) and the most elevated power to reduce iron (FRAP, Figure 4C) was observed

in melanoidins isolated from biscuits (which had the darkest colour and the highest browning index), followed by soft bread and then common bread melanoidins in both ABTS and FRAP methods. Curiously, DPPH assay showed higher antiradical activity in common bread when compared to soft bread. In general, the results reinforce studies about the antioxidant activity of cereal products (Martin et al., 2009, Pastoriza & Rufián-Henares, 2014; Shen, Chen, Li & Tebben, 2019) and are in line with previous works that have established a positive correlation between the browning degree and the antioxidant properties of MR products in honey (Brudzynski & Miotto, 2011b), muffins (Gonzalez-Mateo, Gonzalez-Sanjose & Muñiz, 2009), beer (Martinez-Gomez Caballero & Blanco, 2020), and milk (Calligaris, Manzocco, Anese & Nicoli, 2004). In this sense, Morales and Jiménez-Pérez (2004) isolated melanoidins from different kinds of beer, coffee and sweet wine, and demonstrated that the higher their absorbance at 420 nm, the greater their peroxyl radical scavenging activity. They concluded that this free radical scavenging property is mainly exerted by chromophore groups responsible for brown colour which are linked to the melanoidin skeleton (Morales & Jiménez-Pérez, 2004).

2.5.Cell viability assays

Although melanoidins from coffee and biscuits have shown not to be cytotoxic (Goya, Delgado-Andrade, Rufian-Henares, Bravo & Morales, 2007; Martin et al., 2009), it is imperative to note that some compounds produced during MR induce detrimental effects, including moderate cytotoxicity, which cannot be ignored (Mottram, Low & Elmore, 2006, Muscat, Pischetsrieder, Maczurek, Rothemund & Münch, 2009). The biological activities of melanoidins may be attributed to the degradation product of melanoidins by gastrointestinal digestion and by microbiota fermentation. Several researches have evaluated the degradation of high molecular weight melanoidins by the microbiota of large intestine (Borrelli & Flogiano, 2005, Perez-Burillo, Rajakaruna, Pastoriza, Paliy & Angel Rufian-Henares, 2020), however only a few studies were focused on gastrointestinal digestion effect of melanoidins from bakery products. In this

regard, gastrointestinal digestion of the melanoidins can release healthy or putatively harmful compounds, which can be absorbed or exert their biological action in the site (Wang, Qian & Yao, 2011, Nooshkam, Varidi & Verma, 2020). As this is a preliminary study to characterize melanoidins that will be potentially used in the future as a functional ingredient, the effects of the melanoidin extracts on cell survival were assessed to ensure non-cytotoxic effects of the extracts and their bioaccessible products after *in vitro* gastrointestinal digestion. The concentration of melanoidins assayed (25, 50, 100 and 200 μg/ml) are based in the high intake of melanoidins from Western diet (Fogliano & Morales, 2011).

Caco-2 cells are commonly used in nutrition related studies as they exhibit many characteristics of intestinal epithelium which is primarily exposed to dietary components. On the other hand, endothelial cells, which are highly sensitive to oxidative stress resulting in disturbances of cardiovascular homeostasis, might also benefit from the antioxidant activity of melanoidins (Cossu et al., 2012). For such reasons, Caco-2 and HUVEC cells were tested for viability assays in order to have a representative example of intestinal and endothelial cells respectively. The results in Figure 5A, corresponding to the viability of Caco-2 cells incubated with the three melanoidins extracts, showed non-cytotoxic effect at all the concentration studied. Identical non-cytotoxic effects were observed in Caco-2 cells incubated with the gastro-intestinal digested melanoidins (Figure 5C). These results indicated no significant cytotoxic effect in Caco-2 cells when incubated with soft bread, common bread and biscuit melanoidins themselves, or with their bioaccessible fractions obtained after gastro-intestinal digestion. These data confirm previously reported results where Borrelli et al. (2003) found no cytotoxic effect of melanoidins from biscuits and gluten–glucose model systems bread crust on Caco-2 cells. Similar results were obtained in the hepatic line cell HepG2 incubated with 0.5-10 μg/mL of gastro-intestinal digested biscuit-melanoidins of different molecular weight (Martin et al., 2009).

In the same way, no effect was seen on HUVEC cell viability when incubated with soft bread melanoidins (Figure 5B), or their bioaccessibles fractions (Figure 5D), at any of the studied concentrations. Nevertheless, although cell viability was not affected in HUVEC incubated with 25 and 50 µg/ml of common bread melanoidins, a reduction in HUVEC cell viability was observed in the presence of 100 and 200 µg/ml common bread melanoidins when compared to control (Figure 5B), indicating certain dose-dependent loose of viability. Curiously, when melanoidins were submitted to *in vitro* gastro-intestinal digestion, the decrease in cell viability was only seen at the highest dose of common bread bioaccessible melanoidins (Figure 5D). Finally, incubation of HUVEC cells with biscuit melanoidins at any of the studied concentrations lead to a weak but significant decrease in cell viability with respect to non treated control (Figure 5B). Despite this, gastrointestinal digestion of common bread melanoidins reverted the changes observed in HUVEC cell viability, even at high concentrations (Figure 5D). However, in neither case the decrease of viability was lower to 80 %, indicating not cytotoxic effect by the different products.

These data contrast with previous studies where prune and apricot melanoidins did not affect viability of another endothelial cell line (Cossu et al., 2012, Posadino et al., 2011). The differences are probably due to the cell line or the origin of the melanoidins. In this sense, the present study has demonstrated that the effect on HUVEC cells viability is different depending on the bakery product. However, it is clear that a change occurred during gastrointestinal digestion that made melanoidins safer for endothelial cells. Future studies will be necessary to assess such changes.

3. Conclusion

The “quality” of biscuit melanoidins extracts (considered as the real content of melanoidins and their antioxidant capacity) is substantially higher than these of soft bread melanoidins and far superior than those of common bread extracts. However, a more sustainable alternative to the use of biscuits might be the use of soft bread by-products which besides to offer a higher throughput in the isolation of melanoidins, its extracts were safer for both Caco-2 and HUVEC cells and they remained with a high antioxidant capacity. Additionally, using waste products generated by bakery industry (like the crusts removed from bread) reduces the costs of obtaining melanoidins and provide a sustainable management of bakery by-products. The present study has demonstrated that melanoidins extracted from soft bread crust have a great potential as bioactive compounds due to their antioxidant properties, and might be good candidates for their incorporation to the manufacture of functional bakery products. Future studies are necessary to shed more light on the possible use of melanoidins from bakery by-products as functional ingredients that might be incorporated to foods and make them healthier as well as their effect on organoleptic properties of food formulations.

Credit authorship contribution statement

Noelia Diaz-Morales: Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **M Dolores Rivero-Pérez**: Methodology, Investigation, Validation, Writing - review & editing. **Mónica Cavia-Saiz**: Methodology, Investigation, Validation, Writing - review & editing. **Gonzalo Salazar**: Methodology, Investigation, Resources, Writing - review & editing. **Pilar Muñiz**: Supervision, Project administration, Funding acquisition, Conceptualization, Validation, Investigation, Resources, Methodology, Writing - review & editing.

Acknowledgements

The authors acknowledge financial support from Autonomous Government of Castilla y León and FEDER (JCyL/FEDER) BU243P18; Cerealto Siro Foods (Venta de Baños, Palencia, Spain) for the supply of soft bread by-products (crust) obtained; IMIB-Arrixaca/University of Murcia, Murcia, Spain for kindly supply the HUVEC EA.hy926 cell line.

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.

References

Alves, G., Xavier, P., Limoeiro, R., & Perrone, D. (2020). Contribution of melanoidins from heat-processed foods to the phenolic compound intake and antioxidant capacity of the Brazilian diet. *Journal of food science and technology*, 57(8), 3119-3131. https://doi.org/10.1007/s13197-020-04346-0

Borrelli, R. C., & Fogliano, V. (2005). Bread crust melanoidins as potential prebiotic ingredients. *Molecular nutrition & food research*, 49(7), 673-678. https://doi.org/10.1002/mnfr.200500011.

Borrelli, R. C., Mennella, C., Barba, F., Russo, M., Russo, G. L., Krome, K., Erbersdobler, H. F., Faist, V., & Fogliano, V. (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, 41(10), 1367-1374. https://doi.org/10.1016/s0278-6915(03)00140-6.

Borrelli, R. C., Visconti, A., Mennella, C., Anese, M., & Fogliano, V. (2002). Chemical characterization and antioxidant properties of coffee melanoidins. *Journal of Agricultural and Food Chemistry*, 50(22), 6527-6533. https://doi.org/10.1021/jf025686o.

Brudzynski, K., & Miotto, D. (2011a). The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. *Food Chemistry*, 125(2), 570-575. https://doi.org/10.1016/j.foodchem.2010.09.049.

Brudzynski, K., & Miotto, D. (2011b). The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. *Food Chemistry*, 124(3), 869-874. https://doi.org/10.1016/j.foodchem.2010.07.009.

Calligaris, S., Manzocco, L., Anese, M., & Nicoli, M. C. (2004). Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. *International Dairy Journal*, 14(5), 421-427. https://doi.org/10.1016/j.idairyj.2003.10.001.

Celik, E. E., Rubio, J. M. A., Andersen, M. L., & Gokmen, V. (2018). Interactions of coffee and bread crust melanoidins with hydroxycinnamic and hydroxybenzoic acids in aqueous radical environment. *Food research international (Ottawa, Ont.)*, 108, 286-294. https://doi.org/10.1016/j.foodres.2018.03.057.

Cho, J., Bae, H., Cho, B., & Moon, K. (2017). Qualitative properties of roasting defect beans and development of its classification methods by hyperspectral imaging technology. *Food Chemistry*, 220, 505-509. https://doi.org/10.1016/j.foodchem.2016.09.189.

Cossu, A., Posadino, A. M., Giordo, R., Emanueli, C., Sanguinetti, A. M., Piscopo, A., Poiana, M., Capobianco, G., Piga, A., & Pintus, G. (2012). Apricot melanoidins prevent oxidative endothelial cell death by counteracting mitochondrial oxidation and membrane depolarization. *PloS one*, 7(11), e48817. https//doi.org/10.1371/journal.pone.0048817.

Del Pino-Garcia, R., Garcia-Lomillo, J., Rivero-Perez, M. D., Gonzalez-SanJose, M. L., & Muniz, P. (2015). Adaptation and Validation of QUick, Easy, New, CHEap, and Reproducible (QUENCHER) Antioxidant Capacity Assays in Model Products Obtained from Residual Wine Pomace. *Journal of Agricultural and Food Chemistry*, 63(31), 6922-6931. https//doi.org/10.1021/acs.jafc.5b01644.

Echavarría, A. P., Pagán, J., & Ibarz, A. (2012). Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity. *Food Engineering Reviews*, 4(4), 203-223. https://doi.org/10.1007/s12393-012-9057-9.

Fogliano, V., & Morales, F. J. (2011). Estimation of dietary intake of melanoidins from coffee and bread. *Food & function*, 2(2), 117-123. https://doi.org/10.1039/c0fo00156b.

Gonzalez-Mateo, S., Gonzalez-Sanjose, M. L., & Muniz, P. (2009). Presence of Maillard products in Spanish muffins and evaluation of colour and antioxidant potential. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 47(11), 2798-2805. https://doi.org/10.1016/j.fct.2009.08.015.

Goulas, V., Nicolaou, D., Botsaris, G., & Barbouti, A. (2018). Straw Wine Melanoidins as Potential Multifunctional Agents: Insight into Antioxidant, Antibacterial, and Angiotensin-I-Converting Enzyme Inhibition Effects. *Biomedicines*, 6(3), https://doi.org/10.3390/biomedicines6030083.

Goya, L., Delgado-Andrade, C., Rufian-Henares, J. A., Bravo, L., & Morales, F. J. (2007). Effect of coffee melanoidin on human hepatoma HepG2 cells. Protection against oxidative stress induced by tert-butylhydroperoxide. *Molecular nutrition & food research*, 51(5), 536-545. https://doi.org/10.1002/mnfr.200600228

International Commission on Illumination (2004). *Colorimetry*. (3rd ed.): Commission Internationale de l'Eclairage.

Iriondo-DeHond, A., Ramírez, B., Escobar, F. V., & Castillo, M. D. D. (2019). Antioxidant properties of high molecular weight compounds from coffee roasting and brewing byproducts. *Bioactive Compounds in Health and Disease*, 2(3), 48-63. https://doi.org/10.31989/bchd.v2i3.588.

Kang, O. J. (2016). Evaluation of Melanoidins Formed from Black Garlic after Different Thermal Processing Steps. *Preventive nutrition and food science*, 21(4), 398-405. https://doi.org/10.3746/pnf.2016.21.4.398.

Lindenmeier, M., Faist, V., & Hofmann, T. (2002). Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. *Journal of Agricultural and Food Chemistry*, 50(24), 6997-7006. https://doi.org/10.1021/jf020618n.

Martin, M. A., Ramos, S., Mateos, R., Rufian-Henares, J. A., Morales, F. J., Bravo, L., & Goya, L. (2009). Biscuit melanoidins of different molecular masses protect human HepG2 cells against oxidative stress. *Journal of Agricultural and Food Chemistry*, 57(16), 7250-7258. https://doi.org/10.1021/jf9006032.

Martinez-Gomez, A., Caballero, I., & Blanco, C. A. (2020). Phenols and Melanoidins as Natural Antioxidants in Beer. Structure, Reactivity and Antioxidant Activity. *Biomolecules*, 10(3), 400. https://doi.org/10.3390/biom10030400

Mesías, M., & Delgado-Andrade, C. (2017). Melanoidins as a potential functional food ingredient. *Current Opinion in Food Science*, 14, 37-42. https://doi.org/10.1016/j.cofs.2017.01.007.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Menard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & function*, 5(6), 1113-1124. https://doi.org/10.1039/c3fo60702j.

Morales, F. J., & Jiménez-Pérez, S. (2004). Peroxyl radical scavenging activity of melanoidins in aqueous systems. *European Food Research and Technology*, 218(6), 515-520. https://doi.org/10.1007/s00217-004-0896-3

Mottram, D. S., Low, M. Y., & Elmore, J. S. (2006). 1 - The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods. , 3-22. https://doi.org/10.1533/9781845692018.1.3.

Muscat, S., Pischetsrieder, M., Maczurek, A., Rothemund, Sven, & Münch, G. (2009). Cytotoxicity of Maillard reaction products determined with a peptide spot library. *Molecular Nutrition & Food Research*, 53(8), 1019-1029. https://doi.org/10.1002/mnfr.200800366.

Nooshkam, M., Varidi, M., & Verma, D. K. (2020). Functional and biological properties of Maillard conjugates and their potential application in medical and food: A review. *Food Research International*, 131, e109003. https://doi.org/10.1016/j.foodres.2020.109003.

Oracz, J., & Zyzelewicz, D. (2019). In Vitro Antioxidant Activity and FTIR Characterization of High-Molecular Weight Melanoidin Fractions from Different Types of Cocoa Beans. *Antioxidants (Basel, Switzerland)*, 8(11), e560. https://doi.org/10.3390/antiox8110560.

Ortega-Heras, M., & González-Sanjosé, M. L. (2009). Binding capacity of brown pigments present in special Spanish sweet wines. *LWT - Food Science and Technology*, 42(10), 1729-1737. https://doi.org/10.1016/j.lwt.2009.04.001.

Passos, C. P., Kukurová, K., Basil, E., Fernandes, P. A. R., Neto, A., Nunes, F. M., Murkovic, M., Ciesarová, Z., & Coimbra, M. A. (2017). Instant coffee as a source of antioxidant-rich and sugar-free coloured compounds for use in bakery: Application in biscuits. *Food Chemistry*, 231, 114-121. https://doi.org/10.1016/j.foodchem.2017.03.105.

Pastoriza, S., & Rufián-Henares, J. A. (2014). Contribution of melanoidins to the antioxidant capacity of the Spanish diet. *Food Chemistry*, 164, 438-445. https://doi.org/10.1016/j.foodchem.2014.04.118.

Patrignani, M., Rinaldi, G. J., Rufian-Henares, J. A., & Lupano, C. E. (2019). Antioxidant capacity of Maillard reaction products in the digestive tract: An in vitro and in vivo study. *Food Chemistry*, 276, 443-450. https://doi.org/10.1016/j.foodchem.2018.10.055.

Perez-Burillo, S., Rajakaruna, S., Pastoriza, S., Paliy, O., & Angel Rufian-Henares, J. (2020). Bioactivity of food melanoidins is mediated by gut microbiota. *Food Chemistry*, 316, 126309. https://doi.org/ 10.1016/j.foodchem.2020.126309.

Posadino, A. M., Cossu, A., Piga, A., Madrau, M. A., Del Caro, A., Colombino, M., Paglietti, B., Rubino, S., Iaccarino, C., Crosio, C., Sanna, B., & Pintus, G. (2011). Prune melanoidins protect against oxidative stress and endothelial cell death. *Frontiers in bioscience (Elite edition)*, 3, 1034-1041. https://doi.org/10.2741/e309.

Purlis, E. (2010). Browning development in bakery products – A review. *Journal of Food Engineering*, 99(3), 239-249. https://doi.org/10.1016/j.jfoodeng.2010.03.008.

Roncero-Ramos, I., Delgado-Andrade, C., Haro, A., Ruiz-Roca, B., Morales, F. J., & Navarro, M. P. (2013). Effects of dietary bread crust Maillard reaction products on calcium and bone metabolism in rats. *Amino acids*, 44(6), 1409-1418. https://doi.org/10.1007/s00726-011-1160-3.

Shen, Y., Chen, G., Li, Y., & Tebben, L. (2019). Effect of amino acids on Maillard reaction product formation and total antioxidant capacity in white pan bread. *International journal of food science & technology*, 54(4), 1372-1380. https://doi.org/ 10.1111/ijfs.14027.

Wang, H., Qian, H., & Yao, W. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*, 128(3), 573-584. https://doi.org/10.1016/j.foodchem.2011.03.075.

Yam, K. L., & Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Journal of Food Engineering*, 61(1), 137-142. https://doi.org/10.1016/S0260-8774(03)00195-X.

Figure Captions

Figure 1. CIELAB colour parameters on the crust of the three bakery products. (A) Lightness; (B) Redness/Greeness; (C) Yellowness/Blueness. Different letters indicate significant differences among values ($p < 0.05$) when compared by means of one-way ANOVA followed by Tukey's multiple comparison post-hoc test.

Figure 2. Extraction throughput of melanoidins from the crust of soft bread, common bread and biscuits. Different letters indicate significant differences among values ($p < 0.05$) when compared by means of one-way ANOVA followed by Tukey's multiple comparison post-hoc test

Figure 3. Absorbance values of melanoidins isolated from the crust of three different bakery products when diluted to a final concentration of 3.33 mg/ml in water; and CIELAB colour parameters of freeze-dried powder melanoidins. (A) Melanoidin content assessed as absorbance at 345 nm; (B) Pre-melanoidin content evaluated at 360 nm; (C) Browning index at 420 nm; (D) Lightness; (E) Redness/Greeness; (F) Yellowness/Blueness;. Different letters indicate significant differences among values ($p < 0.05$) when compared by means of one-way ANOVA followed by Tukey's multiple comparison post-hoc test.

Figure 4. Antioxidant activity of melanoidins isolated from the crust of soft bread, common bread and biscuits evaluated with (A) ABTS, (B) DPPH, and (C) FRAP assays. Different letters indicate significant differences among values ($p < 0.05$) when compared by means of one-way ANOVA followed by Tukey's multiple comparison post-hoc test. TE: TROLOX equivalents.

Figure 5. Cell viability relative to untreated control cells of colonic (Caco-2) and endothelial (HUVEC) cell lines following the incubation with different concentrations of (A, B) melanoidins isolated from soft bread, common bread and biscuits; and (C, D) their soluble gastro-intestinal digested fraction. Asterisks indicate significant differences *vs* untreated control ($p < 0.05$) when compared by means of one-way ANOVA followed by Dunnet's multiple comparison post-hoc test. C: Untreated control.

Highlights

- Within bakery products, soft bread has the highest yield in melanoidins extraction
- Melanoidins from biscuits and soft bread crust present a high antioxidant capacity
- Soft bread melanoidins & their bioaccessible fractions do not affect cell viability
- Soft bread crust by-products are safe and sustainable sources of melanoidins
- Melanoidins from soft bread crust by-products are potential functional ingredients

Credit authorship contribution statement

Noelia Diaz-Morales: Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **M Dolores Rivero-Pérez**: Methodology, Investigation, Validation, Writing - review & editing. **Mónica Cavia-Saiz**: Methodology, Investigation, Validation, Writing - review & editing. **Gonzalo Salazar**: Methodology, Investigation, Resources, Writing - review & editing. **Pilar Muñiz**: Supervision, Project administration, Funding acquisition, Conceptualization, Validation, Investigation, Resources, Methodology, Writing - review & editing.