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Bread melanoidins as potential new sustainable bakery ingredients: a study using fat and fat-free bakery food models†

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Melanoidins isolated from bakery by-products are proposed as new sustainable ingredients for bakery products. The colour, odour profile, texture, water activity, and antioxidant capacity of two bakery food models, fat and fat-free, enriched with 2% and 4% soft bread and common bread melanoidins, were analysed. The colour of the bakery food models with melanoidins was darker than that of the respective control; the fat-free models with melanoidins showed higher values of hardness than the control, while no significant effect was observed in the fat models; the water activity did not change compared to the control; the odour profile was significantly modified with different effects depending on the type of melanoidin quantity added and the food model (fat or fat-free); and the antioxidant capacity increased proportionally to the quantity of melanoidin added. In general, melanoidins from soft bread exhibited a higher effect than the melanoidins from common bread. The melanoidins isolated from both fat and fat-free bakery food models did not show cytotoxicity nor did they modify the levels of reactive oxygen species in Caco-2 cells. Therefore, the results seem to indicate the favourable potential of bread melanoidins as new sustainable ingredients for bakery products.

1. Introduction

Food waste is a global problem, and bread is one of the most commonly wasted food products in the countries of the developed world. Bread waste is produced during the manufacturing stage due to processing factors, faults during the operation, rejection during the quality control, and improper handling during the storage/packing, among others.[1] Furthermore, to respond to the consumer demands associated with specific bakery products such as soft bread, bakery companies remove the crusts from loaves, losing up to 40% of the bread[2] and generating a large amount of waste that needs adequate management.

The crusts of bakery products contain melanoidins, brown-coloured pigments of a high molecular weight, which have a high value, both for their technological properties and for their effect on health.[3,4] Melanoidins are formed in the last stages of the Maillard reaction (MR), which takes place during the cooking, thermal processing, and storage of foods, and show a high degree of complexity with an unfamiliar structure.[5] Melanoidins' structure and composition change depending on the food; for instance, in bread, melanoidins are formed by gluten proteins and starch cross-linked by the Maillard reaction products.[6] Melanoidins have drawn a lot of attention owing to their positive effects on the texture and flavour of many food products and, therefore, they are currently used as functional food ingredients in the food industry.

From a technological point of view, melanoidins have a great impact on the sensory quality of food products and contribute to achieving pleasant attributes for consumers, since they provide sensory properties such as colour, texture, and aroma.[7,8] From a health point of view, melanoidins have biological properties with positive health effects, such as chemopreventive, antioxidant, and antimicrobial activities.[5,9,10] European diets include a substantial melanoidin intake of around 10–12 g per day;[11,12] bakery products are significant contributors to this intake. In fact, average amounts of mela-

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noidins from bread and biscuit consumption have been estimated to be 1.8–15 g and 3.2–8.5 g per day, respectively.[11]

The promising use of crust melanoidins obtained from soft and common bread as food ingredients for bread has recently been demonstrated. These melanoidins have high antioxidant capacity, antimicrobial activities on spoilage microbiota and food pathogens, a positive volatile profile, no cytotoxic effects, and no undesirable compounds (furfural and 5-hydroxymethyl-furfural).[13,14] In this regard, it has been suggested that the addition of melanoidins to bakery foods can improve their sensory characteristics.

Based on the previous considerations, the present study has been conducted to revalue the by-products obtained from bakery companies, by isolating melanoidins from the industrial crust residues and using them for reincorporation in other bakery products. In this sense, the present study evaluated the effect of the incorporation of melanoidins in two models of bakery food matrices (with fat and fat-free), since it is hypothesized that they will have a favourable influence on the technological (colour, texture, and aroma) and functional (antioxidant) characteristics of the products.

2. Materials and methods

2.1. Melanoidin separation

The crusts of soft and common bread were used in this study. A Spanish bakery company (Cerealto Foods, Spain) kindly supplied the samples of soft bread crust obtained from processing crustless soft white bread. The common bread was subjected to expansion in a bakery pilot plant, as indicated in a previous study,[13] where the process used to obtain melanoidins from the bread crusts is also indicated. Briefly, the crust samples were ground in a coffee mill (Taurus Minimoka GR20, Taurus Group, Oliana, Spain) and sieved to a particle size <1 mm and melanoidins were extracted by *in vitro* digestion using Pronase E according to the method described by Roncero-Ramos *et al.* (2013).[15] After digestion, the soluble fraction was freeze-dried (FreeZone 12 L Console Freeze Dry System with a drying chamber, Labconco, MO, USA) to obtain the isolated melanoidins as powder products. Melanoidins from the bakery model matrices were also obtained by the same procedure.

2.2. Expansion of the bakery food models supplemented with bread-melanoidins

Two models of bakery food matrices (fat and fat-free) were prepared according to the recipe described in the AACC method.[16] Control formulations (FC and FFC) were subjected to expansion according to the following recipe: wheat flour (12.5 g); sucrose (finely granulated, 5.25 g); and water (6.25 mL). In addition to this, all-purpose vegetable shortening (5 g) was added to the fat control cookies (FC). Isolated melanoidins from common and soft bread crusts were added at 2% or 4% according to the formulation. Thus, ten formulations were subjected to expansion (5 formulations with fat and 5 formulations without fat). The fat cookie formulations were: without melanoidins (FC); with 2% common bread melanoidins (F2-CBM) added; with 4% common bread melanoidins (F4-CBM) added; with 2% soft bread melanoidins (F2-SBM) added; and with 4% soft bread melanoidins (F4-SBM) added. The fat-free cookie formulations were: without melanoidins (FFC); with 2% common bread melanoidins added (FF2-CBM); with 4% common bread melanoidins added (FF4-CBM); with 2% soft bread melanoidins (FF2-SBM) added; and with 4% soft bread melanoidins added (FF4-SBM). The ingredients were mixed and kneaded (KitchenAid Ultra Power KSM90, USA) and the dough was divided into equal portions, with stainless cookie moulds 3 cm in diameter and 0.5 cm thick. Cookies were baked in an electric convection oven (Berto's, Padova, Italy) at 200 ± 5 °C for 15 minutes. Five cookies were obtained for each formulation.

2.3. Colour analysis

The colour of the bakery food models was evaluated directly on the two surfaces of each sample (top and bottom), measuring the CIELab parameters such as lightness (L^*), redness (a^*), and yellowness (b^*). The colour readings were taken at three randomly selected non-overlapping points on the surface of each sample. A Konica Minolta CM-2600d (Konica Minolta Business Technologies Inc., Tokyo, Japan) with the standard illuminant D65 and the standard observer angle 10° (International Commission on Illumination, 2004) was used.

The colour of the isolated powder melanoidins was measured similarly after placing the powders in white paper capsules with an approximate area of 3 cm^2.

The total colour difference among the products and the control was calculated from the CIELab* parameters according to the following formula:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where ΔE^* = color difference, L^*, a^*, and b^* are the values of the processed sample, and L_0^*, a_0^*, and b_0^* are the values of the control sample, respectively.

Furthermore, the absorbance values at 345 and 420 nm of the melanoidin solutions were also considered, being the complementary indexes of the colour. Melanoidins extracted from the bakery food matrices were dissolved in Milli-Q water at a concentration of 10 mg mL^{-1} to prepare an initial solution, which was then diluted at a ratio of 1 : 3 to obtain the working solution of the samples.[13] A spectrophotometer (U-2000 Hitachi, Ltd, Hubbardston, MA, USA) and 1 cm-path length cuvettes were used. Measurements were performed in triplicate (three different working solutions).

2.4. Texture analysis

A puncture test was performed using a TA.XTPlus Texture Analyzer (Stable Microsystems Ltd, Surrey, UK) using the following conditions: test mode, puncture; pre-test speed, 1 mm s^{-1}; test speed, 0.5 mm s^{-1}; post-test speed, 10 mm s^{-1}: trigger force, 5 g. A cylindrical flat-end puncture probe *P*/2 (2 mm diameter) was used. Each sample was centred on the base plate

and the puncture probe penetrated 2 mm into the sample. The texture measurement of the samples involved plotting force (N) *versus* time (s) and two parameters were calculated: (1) area under the curve (as N s) up to 2 mm of puncturing; and (2) the maximum force (N) as an index for the crust hardness. The texture parameters of the bakery food matrices were measured in triplicate (three different pieces). Texture Expert software version 1.2 was used for data analysis.

2.5. Water activity analysis

Water activity values were measured using an AquaLAB CX-2 (Decagon Devices Inc., Pullman, WA, USA). Duplicate measurements from the ground bakery food matrices were carried out.

2.6. Odour profile analysed using an electronic nose

The odour profile of the bakery food models was determined in quintuplicate using an α-FOX 4000 electronic nose (AlfaMOS, Toulouse, France) equipped with 18 metal oxide sensors, and a static headspace system (HS100 CTC-Combi-Pal, CTC Analytics AG, Zwingen, Switzerland). Briefly, the analytical protocol was as follows: glass vials (10 mL) containing 1 g of each ground sample, sealed with Teflon septum taps, were incubated for 10 minutes at 50 °C, and then volatiles were injected into the headspace (2 mL s^{-1} of air), and the sensor responses were collected during 120 s. The response intensity was calculated as the change in the resistance of each sensor with respect to the resistance at time zero. For this work, the maximum values of the response intensity were considered.

2.7. Antioxidant capacity analysis

Quencher methods, ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing power assay), were performed following the procedure described by Del Pino-García *et al.* (2015)[17] with slight modifications. Then, 20 mg of the bakery food model and 6 mL of the ABTS solution; 80 mg of the bakery food model and 3 mL of the DPPH solution; and 10 mg of the bakery food model and 6 mL of the TPTZ solution were used. After incubation with continuous shaking for 10 minutes and centrifugation at 10 500*g* for 3 minutes, the absorbance of the supernatants (reactive solutions) was measured at the respective wavelength. The antioxidant capacity was measured in quintuplicate.

2.8. Cell studies

Human colon adenocarcinoma Caco-2 (ATCC® HTB-37™) cells were purchased from the American Type Culture Collection (ATCC, Barcelona, Spain). The cells were cultured as a monolayer in Eagle's Minimum Essential Medium (MEM) supplemented with 20% v/v heat inactivated fetal bovine serum (FBS), 1% (v/v) non-essential amino acids, 1% (v/v) L-glutamine, 1% (v/v) sodium pyruvate, and 1% (v/v) penicillin/streptomycin. The cell cultures were incubated at 37 °C and 90% humidity under a 5% CO_2 atmosphere.

2.8.1. Cytotoxicity. The cytotoxicity of melanoidins was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[18] The cells were seeded in 96-well microplates at a density of 1×10^4 cells per well, and pre-incubated for 24 h to allow them to attach and proliferate. Melanoidins extracted from the bakery food models were dissolved in phosphate buffered saline (PBS) solution (pH 7.4) and sterile filtered. The cells were exposed to different concentrations of melanoidins (50, 100, and 200 μg mL^{-1}) in a complete culture medium for 24 h. After the treatment, MTT solution (5 mg mL^{-1} in phosphate buffered saline, PBS) was added and incubated for 120 min at 37 °C. The absorbance at 570 nm was measured using a PowerWave XS2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The results were expressed as the percentage of viable cells compared to non-treated cells (control).

2.8.2. Intracellular reactive oxygen species (ROS) measurement. An intracellular ROS scavenger assay was performed by measuring the fluorescence intensity of the 2′,7′-dichlorodihydro-fluorescein diacetate (DCFH-DA) probe, which is proportional to the amount of ROS formed.[19] For the experiments, 24 h after the treatment with the melanoidin fractions (50 μg mL^{-1}), the cells were incubated with 20 μM DCFDA for 30 minutes at 37 °C. After incubation, the intracellular DCF fluorescence of cells not treated (NT) and cells treated with melanoidins (SM) was evaluated at 485 nm/528 nm using a PowerWave XS2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). Fluorescence emission was recorded after incubation in the dark at 37 °C for 30 min (t_{30}) and the variations in the relative fluorescence units ($t_{30} - t_0$) were calculated for each sample. The experiments were performed in triplicate and the ROS levels were estimated as the DCF fluorescence increased, and the results were expressed as ROS inhibition percentages using the equation: T/C [%] = $\Delta F_{SM(t_{30}-t_0)}/\Delta F_{NT(t_{30}-t_0)}$.

2.9. Statistical analysis

Two-way analysis of variance (ANOVA) was performed, and statistical comparisons of the different treatments were performed using Sidak's multiple comparison *post hoc* test. When comparisons were carried out against the control (this is the case for cell viability assays), Dunnett's multiple comparison test was conducted. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA). For the statistical analysis of the odour profile data, one-way analysis of variance (ANOVA) of the response of the sensors was performed using Tukey's multiple comparison *post hoc* test and the SPSS Statistics version 24 (IBM Corp., New York, NY, USA) package.

3. Results and discussion

The main characteristics of the melanoidins used in this study, such as the absence of cytotoxicity, antimicrobial and

antioxidant properties, colour, and elemental composition, were shown in previous studies.[13,14] According to these studies, it was established that the most suitable concentrations of melanoidins for incorporation in the studied bakery food models were 2% and 4%.

3.1. Effect of bread-melanoidin addition on the quality properties of the bakery food models

The surface colour, texture, and the odour profile are the main features of baking products that are considered as being preferential by consumers; therefore, they are very important for determining the quality of these products. For that reason, this study aimed at analyzing these parameters, together with others directly correlated with them, such as water activity that influences the texture, or antioxidant capacity with a direct effect on odour by delaying rancidity.

3.1.1. Colour of the bakery food models. During baking, the development of colour is due to the Maillard reaction. The typical colour of bakery products ranges from yellow to brown, depending on different factors such as the ingredients used to make the dough, baking temperature and time, and the water activity in the system.[7,20] In general, the surface colour of bakery products is a parameter that determines their quality, texture, aroma, and flavour, and is highlighted as one of the main aspects considered by consumers.[12,20,21]

The effect of common and soft bread melanoidins on the CIELab* colour parameters (Table 1) pointed out that in the case of fat-free bakery matrices, the incorporation of 2% or 4% of common or soft bread melanoidins did not significantly change ($p > 0.05$) the values of the L^*, a^* and b^* parameters. However, the values of these parameters in the case of fat bakery food models were significantly modified ($p < 0.05$). Fat matrices supplemented with melanoidins showed significantly lower values of L^*, which decreased proportionally with the increase in the quantity of the added melanoidins, leading to darker or browner samples. These changes were statistically significant with soft bread melanoidins, which also increased the a^* value (redness). However, component b^* (yellowness) remained constant or decreased. Passos *et al.* (2017)[7] also observed a decrease in the b^* component in cookies after the incorporation of high amounts of coffee melanoidins. The soft bread melanoidins used are rich in melanoidins with a high molecular weight and are relatively dark in colour, corresponding to the intense absorbance at 420 nm (Diaz-Morales *et al.*, 2021).[13] Hence, they are probably responsible for the colour changes observed in the fat bakery food models.

Fat exerts a positive effect on the colour of the bakery products and the absence or reduction of fat renders less coloured or dark products.[22,23] This fact explains the difference between the values of the CIELab* parameters of both model matrices (fat and fat-free) and could also explain why the added melanoidins significantly modified the colour parameters of only the fat matrices.

The parameter "difference in the colour" (ΔE^*) allows estimation of the global colour changes between the two samples. Thus, comparing the food bakery models with their respective controls, a notable global effect of the addition of melanoidins on the colour of the bakery matrices was detected. For all the cases, significant colour differences were observed (ΔE^* values higher than 1 and statistically different from 0). However, in general, values over 5 (the limit value for visual colour discrimination) were detected in the fat model matrices or when soft bread melanoidins were used (Table 1).

From the increase in colour (darkness) observed mainly upon the addition of soft bread melanoidins, it is possible to hypothesize that they could be a good alternative to achieve the desirable toasted colour by applying a low-intensity baking process (low temperature and/or short time) with the corresponding energy reduction and the decrease in the risk of undesirable compounds formed during heating, such as acrylamide.

3.1.2. Texture and water activity of the bakery food models. Melanoidins can induce changes in the texture of the bakery products.[21] These changes have been attributed to the protein

Table 1 Effect of melanoidin incorporation on the colour parameters of the bakery food models

		Fat-free bakery food models					Fat bakery food models				
			Common bread melanoidins		Soft bread melanoidins			Common bread melanoidins		Soft bread melanoidins	
		Control	2%	4%	2%	4%	Control	2%	4%	2%	4%
Top surface	L^*	67 ± 6^{a}	70 ± 2^{a}	66 ± 1^{a}	62 ± 7^{a}	62 ± 7^{a}	$58 \pm 9^{\chi\#}$	$57 \pm 12^{\beta\chi\#}$	$51 \pm 5^{\beta\chi\#}$	$49 \pm 8^{\alpha\beta\#}$	$41 \pm 6^{\alpha\#}$
	a^*	6 ± 3^{ab}	4 ± 1^{a}	6 ± 3^{ab}	8 ± 3^{b}	8 ± 3^{ab}	$9 \pm 3^{\alpha\#}$	$8 \pm 4^{\alpha}$	$13 \pm 2^{\beta\#}$	$13 \pm 1^{\beta\#}$	$13 \pm 1^{\beta\#}$
	b^*	24 ± 2^{a}	24 ± 1^{a}	25 ± 3^{a}	25 ± 3^{a}	26 ± 3^{a}	$30 \pm 2^{\beta\#}$	$28 \pm 2^{\alpha\beta\#}$	$31 \pm 2^{\beta\#}$	$28 \pm 4^{\beta}$	$24 \pm 5^{\alpha}$
	ΔE^*		5 ± 2^{ab}	4 ± 1^{a}	9 ± 2^{c}	7 ± 4^{bc}		$7 \pm 2^{\alpha}$	$12 \pm 4^{\beta\#}$	$6 \pm 1^{\alpha\#}$	$19 \pm 4^{\delta\#}$
Bottom surface	L^*	59 ± 6^{a}	58 ± 5^{a}	56 ± 3^{a}	59 ± 6^{a}	52 ± 5^{a}	$55 \pm 8^{\beta}$	$52 \pm 8^{\beta}$	$37 \pm 9^{\alpha\#}$	$42 \pm 7^{\alpha\#}$	$34 \pm 5^{\alpha\#}$
	a^*	10 ± 4^{a}	11 ± 4^{a}	14 ± 1^{a}	11 ± 3^{a}	13 ± 1^{a}	$10 \pm 5^{\alpha}$	$13 \pm 5^{\alpha\beta}$	$14 \pm 2^{\beta}$	$14 \pm 2^{\beta}$	$13 \pm 2^{\alpha\beta}$
	b^*	30 ± 3^{a}	31 ± 3^{a}	32 ± 1^{a}	31 ± 2^{a}	32 ± 1^{a}	$30 \pm 3^{\chi\delta}$	$32 \pm 2^{\delta}$	$23 \pm 9^{\alpha\beta\#}$	$26 \pm 5^{\beta\chi\#}$	$19 \pm 6^{\alpha\#}$
	ΔE^*		6 ± 3^{a}	6 ± 1^{a}	5 ± 2^{a}	7 ± 3^{a}		$10 \pm 4^{\alpha}$	$21 \pm 9^{\beta\#}$	$12 \pm 6^{\alpha}$	$24 \pm 4^{\beta}$

Different Latin letters indicate significant differences among the formulations of bakery food models without fat ($p < 0.05$). Different Greek letters indicate significant differences among the formulations of bakery food models with fat ($p < 0.05$). # Indicates significant differences between the fat-free and fat models in the same formulation ($p < 0.05$). Comparisons were made by two-way ANOVA, followed by Sidak's multiple comparison test.

crosslink formation, emulsion properties, or protein–polysaccharide conjugate formation.[8]

The incorporation of melanoidins involved some significant changes in the texture of the studied bakery food models, which were mainly dependent on the types of melanoidins added and the presence/absence of fat (Fig. 1A). The obtained results agree with those reported by the other authors, who also pointed out the changes in hardness due to the different Maillard products.[7,24]

The fat-free model matrices added with common bread melanoidins (2% and 4%) showed significant increases in the hardness, whereas no changes were observed in the fat model matrices. Fat provides lubricity to the dough, reduces the formation of the gluten network ("shortening effect"), and is involved in the transfer of heat and expansion during the baking process.[25] All of these effects could explain why, in the presence of fat, the effect of the added melanoidins was not notable.

In contrast with the previous results, the addition of soft bread melanoidins did not involve significant texture changes. The different behaviors of both melanoidins could be due to their different characteristics; some of them were revealed in previous papers.[13,14] A large number of studies on melanoidins, isolated from real foods and model systems, reported the huge variety of their structures and activities, due to the conjunction of factors (ingredients or reactive compounds, heating conditions, and so on) that influence their formation.[12]

Furthermore, from the fact that melanoidins are hygroscopic substances, their effect on the hardness of the fat-free matrices could be correlated with their possible effect on water activity. So, although the addition of melanoidins did not involve significant differences in the water activity of the studied matrices (Fig. 1B), the data showed an increasing tendency when common bread melanoidins were added. Regardless, the obtained water activity values ranged within the interval of a_w of 0.16–0.27, which are typical values for bakery products such as cookies (mean value around 0.2).[26]

3.1.3. Odour profile of the bakery food models. Previous papers noted the role of melanoidins in food aroma, which is mainly due to their capacity to interact and retain small MRPs and other volatiles.[27,28]

The incorporation of melanoidins involved some significant changes in the odour profile (e-nose sensor response) of the studied bakery food models, which were mainly dependent on the types of melanoidins added and the presence/absence of fat (Fig. 2). The data from 4 of 18 sensors were not included in the analysis, due to their low responses (response intensity lower than 1).

The addition of common bread melanoidins, at both 2% and 4%, modified the odour profile of the bakery food models, either with fat or without fat. In general, the incorporation of common bread melanoidins produced significant increases in the maximum response intensity for about half of the sensors, in both types of matrices (fat and fat-free), and then the odour profile was noticeably modified both qualitatively and quantitatively. Conversely, when soft bread melanoidins were added, the odour profiles were quite like those of the control, mainly in the case of fat matrices. In the case of fat-free matrices, the data showed significant quantitative differences (lower intensity response) for most of the sensors.

Considering that the higher the quantity of volatiles in the matrices the higher the response of the sensors, the obtained results can be explained by the different volatile compositions of both melanoidins. A previous study[14] noted that common bread melanoidins are richer in volatile components, and that they showed more volatiles and were in higher quantity compared to soft bread melanoidins. This previous work revealed that while the volatile profile of common bread melanoidins included, among others, superior alcohols, pyrazines, and some lactones, neither of these compounds were detected in soft bread melanoidins. Furthermore, soft bread melanoidins did not show some acids such as butanoic, isovaleric, and octanoic; all of these volatile compounds can interact with sensors and modify their response. The volatile composition of common bread melanoidins could also explain the increase

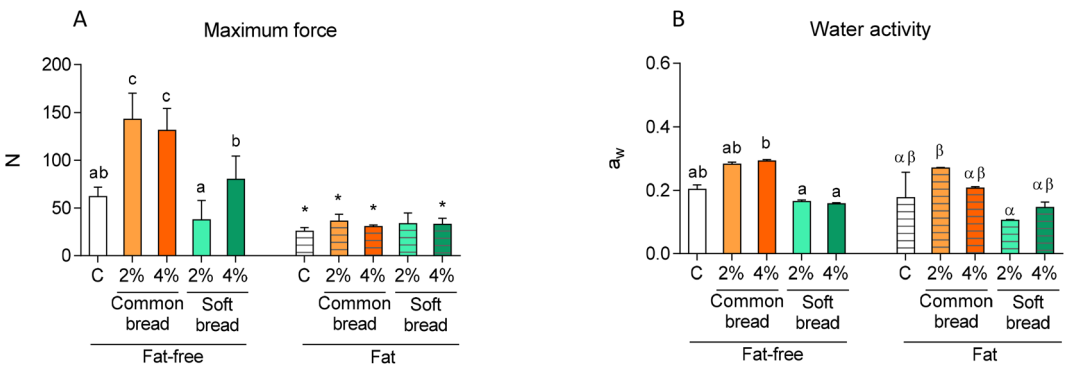

Fig. 1 Effect of melanoidin incorporation on the hardness (A) and water activity (B) of bakery food models. Different Latin letters indicate significant differences among the formulations of the bakery food models without fat ($p < 0.05$). Different Greek letters indicate significant differences among the formulations in the bakery food models with fat ($p < 0.05$). * Indicates significant differences between the fat and fat-free models in the same formulation ($p < 0.05$). Comparisons were made by two-way ANOVA, followed by Sidak's multiple comparison test.

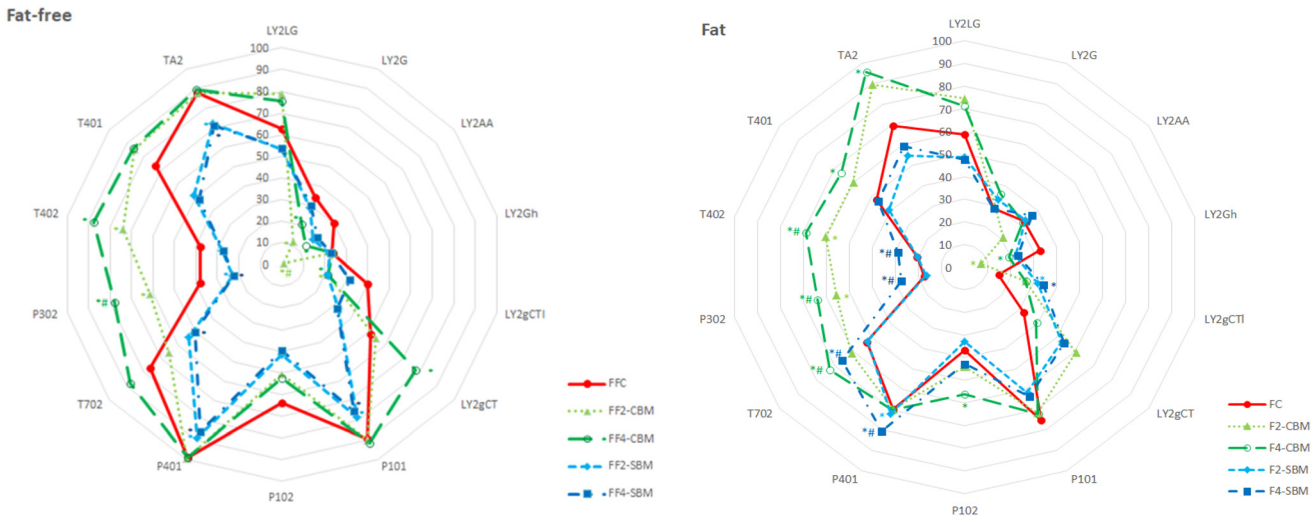

Fig. 2 The odour profile of the bakery food models: control (C), fat-free (FF) and fat (F) with 2% or 4% common bread (CBM) or soft bread (SBM) melanoidins added, evaluated by the response intensity $((R_0 - R)/R_0)$ of 14 sensors using an α-FOX electronic nose. * Indicates significant differences between formulation C and the formulations with CBM or SBM ($p < 0.05$). # Indicates significant differences between the concentrations 2% and 4% CBM or SBM in the bakery food models ($p < 0.05$). Comparisons were made by one-way ANOVA, followed by Tukey's multiple comparison test.

in sensor responses with higher concentrations of these melanoidins, which was not observed in the case of soft bread melanoidins.

3.1.4. Antioxidant capacity of the bakery food models with bread melanoidins. The mechanism of the antioxidant effect of melanoidins is based on their ability to trap positively charged electrophilic species, scavenge oxygen radicals, or carry out metal chelation to form inactive complexes.[29,30]

The incorporation of melanoidins involved some significant changes in the antioxidant capacity of the studied bakery food models, which were dependent on the types of melanoidins added and the presence/absence of fat (Fig. 3). In general, the addition of melanoidins into fat bakery food models involved the most noticeable changes, showing significant and concentration-dependent increases in the antioxidant activity. The results indicate the influence of fat on the antioxidant capacity of the bakery food models, possibly by the liposolubility of melanoidins. Wagner *et al.* (2002)[31] previously described similar results – increases in the antioxidant activity of melanoidins in a fat medium.

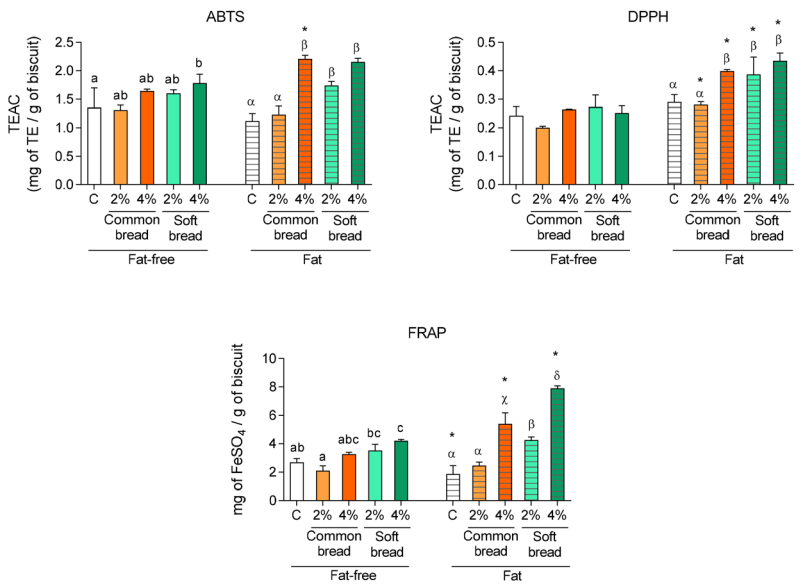

Fig. 3 Effect of the incorporation of melanoidins from common bread and soft bread on the antioxidant capacity of the bakery food matrices (fat-free and with fat). Different Latin letters indicate significant differences among the fat-free model matrices ($p < 0.05$). Different Greek letters indicate significant differences among the matrices with fat ($p < 0.05$). * Indicates significant differences between the fat and fat-free models in the same formulation ($p < 0.05$). Comparisons were made by two-way ANOVA, followed by Sidak's multiple comparison test.

In summary, it seems to be possible to assert that incorporation of bakery melanoidins into bakery products contributes to increasing their antioxidant capacity and corroborates the idea that melanoidins could be used as potential functional food ingredients to increase the shelf-life of bakery products, noted previously by other authors.[5,6]

3.2. Characteristics of the melanoidins formed in the bakery food models enriched with bread melanoidins

From the fact that melanoidin formation depends on, among other factors, the ingredients used to make the bakery products, the hypothesis that melanoidins as an ingredient of the dough probably influence the properties of the new melanoidins formed during the baking process was checked. To check this, the properties of the melanoidins isolated from the studied bakery food models (with and without melanoidins) were compared. To our knowledge, no previous studies have focused on this fact.

Due to the importance of food safety and security, one of the properties evaluated and compared based on the new melanoidins was their cytotoxicity. This fact was under consideration because although melanoidins added in the expansion process of the bakery food models did not show cytotoxicity,[14] the new melanoidins generated in the model matrices could have developed a more complex structure and incorporated cytotoxic compounds that might change the non-toxic characteristics of the initial melanoidins.

No cytotoxicity (decrease of cell viability) was observed at the studied concentrations for Caco-2 cells (Fig. 4). These data agree with the results previously reported, indicating no cytotoxic effect of the different melanoidins isolated from the bakery products.[12,32,33] Furthermore, the new melanoidins did not exhibit prooxidant activity, as there was no increase in the intracellular ROS levels in the presence of melanoidins (Fig. 4). These results agree with those previously reported upon working with melanoidins from bakery and other foods.[34–36] Altogether, the cell viability and intracellular ROS levels indicate that the harmless effect of the initial melanoidins, similar to the new melanoidins from the control model matrices, remains in the new melanoidins formed in the bakery food models supplemented with melanoidins.

The new melanoidins formed in the fat bakery food models were, in general, darker than those of the control model matrices (Table 2), as can be seen from the data for powder melanoidins (L^* and ΔE^*) and from the melanoidin solutions (365 nm and 420 nm). The effect of melanoidins on colour was concentration dependent, and variable with the types of melanoidins added. The darkest melanoidins were those isolated from the model matrix with 4% soft bread melanoidins added. Fat-free bakery food models showed similar results, but with fewer differences in the L^* values. These results could be because the initial SBM were darker compared to the CBM, as observed in previous studies.[13] Regardless, altogether, the colour results pointed out the contribution of the added melanoidins to the colour of the newly formed melanoidins.

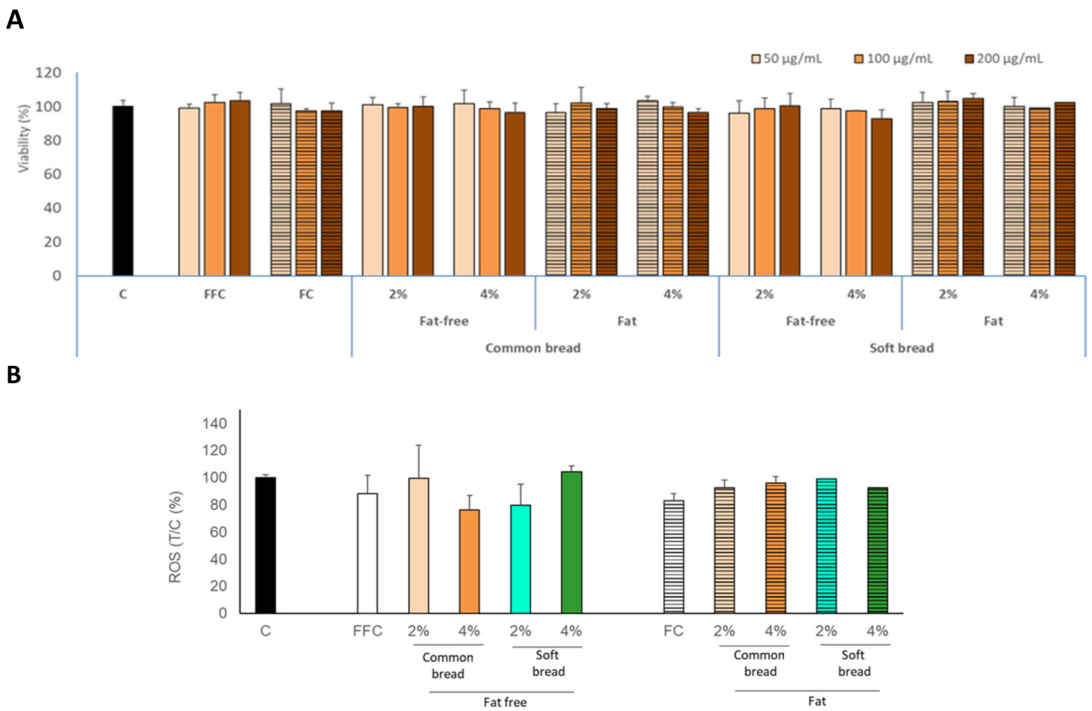

Fig. 4 (A) Caco 2-cell viability in the presence of 50, 100 and 200 µg mL^{-1} of the new melanoidins isolated from the studied bakery food models. (B) The intracellular ROS levels of the Caco-2 cells incubated with 50 µg mL^{-1} of the new melanoidins. C: Untreated cells; FFC: control bakery free-fat matrices; FC: control bakery fat matrices. The bars show the median values and the whiskers indicate the standard deviations. Comparisons were made by two-way ANOVA, followed by Dunnett's multiple comparison test.

Table 2 Some properties of the melanoidins obtained from the bakery model matrices with common and soft bread melanoidins added

Formulation		L^*	a^*	b^*	ΔE^*	365 nm (UA)	420 nm (UA)	ABTS (TEAC)	FRAP, mg g^{-1}
Fat-free model matrices	FFC	69 ± 0.6^{bc}	2 ± 1^{b}	13 ± 0.4^{a}	—	0.123 ± 0.05^{a}	0.039 ± 0.001^{a}	2 ± 0.1^{a}	4 ± 0.3^{b}
	FF2-CBM	64 ± 0.3^{ab}	1 ± 0.1^{a}	14 ± 0.2^{ab}	5 ± 0.3^{c}	0.179 ± 0.01^{c}	0.048 ± 0.001^{b}	3 ± 0.3^{b}	6 ± 1.0^{c}
	FF2-SBM	69 ± 0.1^{bc}	4 ± 0.5^{d}	16 ± 0.2^{cd}	3 ± 0.2^{a}	0.161 ± 0.01^{b}	0.053 ± 0.005^{c}	3 ± 0.2^{ab}	5 ± 0.4^{ab}
	FF4-CBM	73 ± 0.1^{c}	3 ± 0.1^{c}	15 ± 0.1^{bc}	4 ± 0.1^{b}	0.120 ± 0.03^{a}	0.027 ± 0.002^{a}	2 ± 0.4^{ab}	4 ± 0.1^{a}
	FF4-SBM	64 ± 0.2^{a}	4 ± 0.8^{d}	18 ± 0.3^{d}	7 ± 0.4^{d}	0.272 ± 0.03^{d}	0.081 ± 0.001^{d}	3 ± 0.2^{b}	7 ± 0.8^{c}
Fat model matrices	FFC	$68 \pm 0.2^{\delta}$	$3 \pm 0.4^{\alpha}$	$16 \pm 1.4^{\alpha}$	—	$0.253 \pm 0.05^{\alpha}$	$0.110 \pm 0.05^{\beta}$	$2 \pm 0.1^{\beta}$	$5 \pm 0.4^{\alpha}$
	F2-CBM	$69 \pm 0.3^{\varepsilon}$	$3 \pm 0.1^{\alpha}$	$15 \pm 0.1^{\alpha}$	$2 \pm 0.3^{\alpha}$	$0.196 \pm 0.08^{\alpha}$	$0.070 \pm 0.04^{\alpha}$	$2 \pm 0.1^{\alpha}$	$5 \pm 0.5^{\alpha}$
	F2-SBM	$65 \pm 0.4^{\beta}$	$5 \pm 0.1^{\beta}$	$21 \pm 0.2^{\beta}$	$5 \pm 0.2^{\beta}$	$0.357 \pm 0.12^{\chi}$	$0.122 \pm 0.06^{\chi}$	$4 \pm 0.1^{\beta}$	$7 \pm 0.7^{\beta}$
	F4-CBM	$62 \pm 0.2^{\gamma}$	$6 \pm 0.1^{\gamma}$	$22 \pm 0.2^{\beta}$	$9 \pm 0.2^{\chi}$	$0.468 \pm 0.02^{\delta}$	$0.177 \pm 0.02^{\delta}$	$3 \pm 0.2^{\beta}$	$7 \pm 0.7^{\beta}$
	F4-SBM	$57 \pm 0.3^{\alpha}$	$7 \pm 0.1^{\delta}$	$22 \pm 0.2^{\beta}$	$13 \pm 0.3^{\delta}$	$0.786 \pm 0.06^{\varepsilon}$	$0.299 \pm 0.03^{\varepsilon}$	$5 \pm 0.4^{\chi}$	$11 \pm 1.4^{\chi}$

Different Latin letters indicate significant differences among the fat-free model matrices ($p < 0.05$). Different Greek letters indicate significant differences among the matrices with fat ($p < 0.05$). Comparisons were made by two-way ANOVA, followed by Sidak's multiple comparison test. FF: fat-free; F: fat; CBM: common bread melanoidins; SBM: soft bread melanoidins; UA: the unit of absorbance – the antioxidant total capacity evaluated using ABTS and FRAP. TEAC (trolox equivalent antioxidant capacity): mg of Trolox per g melanoidin.

The comparison of the colour results of the new melanoidins and the bakery food models seemed to indicate a direct correlation between them, and then reinforced the idea of a direct impact of melanoidins on the colour of the bakery food models.

The new melanoidins formed in each studied bakery food model showed values of antioxidant activities similar to those obtained previously.[13] These results corroborate the crucial role of melanoidins in preventing oxidative damage due to their antioxidant capacity, including the metal ion chelation capacity and radical-scavenging activity.[5,10,31]

The data from ABTS and FRAP indicated some significant differences (Table 2). However, in general, the results do not allow the assertion that the addition of melanoidins involved significant modification of the antioxidant properties of the new melanoidins formed. Only the melanoidins isolated from the bakery food model with 4% SBM had higher values of antioxidant capacity compared to those isolated from the control matrices, regardless of the presence or absence of fat and the method of analysis to evaluate the antioxidant capacity. In the other cases of this study, the addition of melanoidins did not involve significant changes or these were discordant between the matrices or the doses of melanoidins added. The obtained results seem to have a correlation with the higher antioxidant activity of SBM compared to CBM.[14]

4. Concluding remarks

Melanoidins isolated from different bakery by-products, especially those isolated from the crusts removed from soft bread, are a good candidate for use as "new" ingredients for bakery foods because they significantly improve properties such as colour, texture, and antioxidant capacity, which are directly correlated with the acceptance of consumers and with the global quality of these types of products. Furthermore, the melanoidins formed in the bakery products with the melanoidins added did not show cell cytotoxicity and maintained their antioxidant properties.

Therefore, the isolation of melanoidins from bakery by-products can be an alternative to reuse and revalue these by-products, contributing to the circular economy and the sustainable production of food.

Abbreviations

CBM Melanoidins isolated from common bread
SBM Melanoidins isolated from soft bread

Author contributions

Noelia Díaz-Morales: methodology, investigation, and writing – review and editing. Mónica Cavia-Saiz: methodology, investigation, validation, and writing – review and editing. M. Dolores Rivero-Pérez: methodology and writing – review and editing. Inmaculada Gómez Bastida: methodology, validation, and writing – review and editing. Gonzalo Salazar-Mardones: methodology, investigation, resources, and writing – review and editing. Isabel Jaime Moreno: supervision and writing – review and editing. Maria L. González-SanJosé: conceptualization, supervision, writing – original draft, and writing – review and editing. Pilar Muñiz: conceptualization, supervision, project administration, funding acquisition, resources, writing – original draft, and writing – review and editing.

Conflicts of 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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