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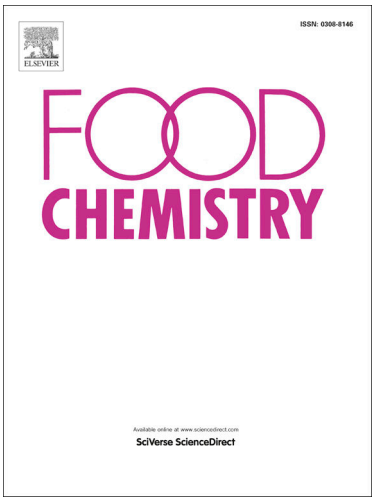

Short communication

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Elucidating the structure of melanoidins derived from biscuits: A preliminary study

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Abstract

Melanoidins present important physiological activities, but their structure is largely unknown. The objective of the present work was to reveal the physicochemical characteristics of biscuit melanoidins(BM) prepared under high temperature(HT) and low temperature(LT) conditions (150°C/25min-100°C/80min respectively). BM were characterised and analysed by differential scanning calorimetry, X-ray and FT-IR. Moreover, the antioxidant capacity and the zeta potential were determined. The phenolic content of HT-BM was higher than that of LT-BM (19.5±2.6% vs 7.8±0.3% respectively, $p \leq 0.05$) and the antioxidant capacity determined by ABTS/DPPH/FRAP ($p \leq 0.05$) was greater. Also, HT-BM presented a 30% increase in crystal structure compared to LT-BM according to X-ray analysis. The magnitude of the negative net charge was significantly higher in HT-BM (-36.8±0.6) than in LT-BM (-16.8±0.1)($p \leq 0.05$). FT-IR analysis confirmed the presence of phenolic and intermediate Maillard reaction compounds bound to the HT-BM structure. In conclusion, the different heating treatments applied to biscuits led to differences in the melanoidin structure.

Keywords

Biscuit melanoidins; structure analysis; crystallinity; negative charge; antioxidant capacity

1. Introduction

Over the last 20 years, the scientific interest in Maillard reaction (MR) has increased due to its nutritional and physiological implications. This non-enzymatic reaction leads to the development of a complex series of molecules including the advanced glycation end products (AGEs) which have been associated with the occurrence of many chronic diseases, such as diabetes, osteoporosis, and Alzheimer's disease (Tian et al., 2023). However, it is still debated whether the thermally treated food has negative consequences for health, mainly because new evidence has indicated that the absorption rate of AGEs is limited in the gastrointestinal tract and because not only AGEs but also beneficial compounds are formed during the heating treatment (Snelson & Coughlan, 2019; Nie et al., 2022). The latter is mainly related to the presence of high molecular weight polymers, better known as melanoidins, which develop at advanced steps of MR (Morales et al., 2012; Patrignani et al., 2019). Although melanoidins were at first considered an inert material, further investigations demonstrated their antioxidant, antimicrobial, anti-inflammatory, antihypertensive and prebiotic activity (Mesías & Delgado-Andrade, 2017).

Unfortunately, the chemical arrangement of melanoidins is not well understood and its link with health benefits is mostly undefined (Rajakaruna et al., 2022). Some authors have used model systems with sugars and amino acids under controlled conditions to study melanoidin structure. However, these are "oversimplified analyses" that do not consider the additional reactants that could also be involved during the development of MR in real food systems. For example, it has been reported that in products such as coffee, bread or biscuits, different constituents (such as phenolic compounds) may become part of the melanoidin structure (Mesías & Delgado-Andrade, 2017), while some new evidence has revealed the presence of lipid components (Rajakaruna et al., 2022) and aromatic structures (Shaheen et al., 2021). Therefore, in order to properly study the melanoidin characteristics and be able to associate them with their health benefits, real food systems should be used.

In a recent study Antonietti et al. (2022) compared the composition and potential biological activity of high molecular weight melanoidins isolated from instant soluble coffee and instant soluble barley. Their results indicated that melanoidins exert an important scavenging activity against radicals, and a high neuroprotective effect that may be associated with the presence of phenolic and ortho-diphenols in their structure. On the other hand, Díaz-Morales et al. (2023) analysed the melanoidins extracted from bread crust, and also confirmed their antioxidant capacity while no cell cytotoxicity was observed. Nonetheless, the structural arrangement of melanoidins extracted from this bakery product was not analysed and requires clarification. According to Nunes et al. (2022) this lack of knowledge limits our understanding of the impact that melanoidins have on nutrition and health.

Biscuits have one of the highest melanoidin contents, which entails an important contribution to the antioxidant activity in a diet (Pastoriza & Rufián-Henares, 2014; Mesías & Delgado-Andrade, 2017). Besides, previous studies performed by our research group demonstrated that the intake of biscuit melanoidins prepared at high temperature (150°C) reduced body weight, increased the antioxidant capacity of serum, had a prebiotic effect and positively affected the antioxidant environment in the gut, and did not reduce the mineral absorption *in vivo* (Patrignani et al., 2016; Patrignani et al. 2019). However, these effects were not observed when biscuits were prepared at low temperature (100°C). Our hypothesis is that the physiological effects of the Maillard reaction products (MRP) account for the different structural arrangements that melanoidins undergo during the heating treatment. Therefore, the objective of the present work was to reveal the molecular structure and the physical characteristics of biscuit melanoidins that could explain their health benefits. In order to do this, reliable physicochemical techniques were applied for the first time to these macromolecules.

2. Materials and methods

2.1 Materials and biscuit preparation

The chemicals used were of analytical grade, and ingredients were of food grade. Biscuits were prepared as described in a previous work (Patrignani et al., 2016) and baked under two different conditions: 150°C/25 min (high temperature, HT) or 100°C/80 min (low temperature, LT) (see Supplementary Data). Patrignani et al. (2016) had previously performed the *in vivo* studies and indicated that these temperatures were suitable to observe the melanoidin development in biscuits.

2.2 Extraction of melanoidins and physicochemical characterisation

The complete description of the melanoidin analysis can be found in the Supplementary Data. Briefly, melanoidins (molecular weight>5 KDa) were isolated by enzymatic hydrolysis and lyophilised (Pérez-Burillo et al., 2020). Their absorbance parameters (K_{294} and K_{420} expressed as L/g.cm), carbohydrate and protein content, antioxidant activity (according to DPPH, FRAP and ABTS), total phenolic content (TPC), differential scanning calorimetry (DSC) analysis, X-ray diffraction (XRD), and infrared spectroscopy spectra (FT-IR) (128 scans for each sample) were determined as described by Patrignani and González-Forte (2021). For the zeta potential (ZP), the pH of the resuspended samples was adjusted to 7, and the determination was performed at 25°C using an SZ-100 nanoparticle analyser (Horiba Ltd., Kyoto, Japan) (five readings for each sample).

2.3 Statistical analysis

All tests and treatments were run in triplicate unless otherwise stated. Results were statistically evaluated by analysis of variance followed by a Fisher's test. In both cases a 0.05 significance level was used (InfoStat 2012, UNC, Córdoba, Argentina).

3. Results and discussion

3.1 Melanoidin content and absorbance parameters

Previous research indicated that the amount of melanoidins increased with the intensity of the heating treatment (Xiang et al., 2020). However, in the present work no significant differences were found in the amount of melanoidins in biscuits cooked at low (100°C, LT-BM) or high (150°C, HT-BM) temperature (6.8% and 7.6%, LT-BM and HT-BM content respectively, $p>0.05$) (Table 1). This result is in good agreement with that reported by Lopes et al. (2016), who indicated that the increase in the browning development was not associated with the amount of melanoidins extracted from different espresso coffees. While Kurniawan et al. (2017) did not find significant differences in the melanoidin content of light, medium and dark robusta or arabica coffee extracts ($p>0.05$). Therefore, these authors also found that the total amount of melanoidins did not increase with the thermal treatment, but it affected the structural arrangements of melanoidins and their functional groups. This idea was also supported by Patrignani and González-Forte (2021) studies, who analysed the melanoidin content and structure of brewer's spent grain. According to their assays, after the setting of the melanoidin structure, further thermal treatment induced changes in their structural arrangements with no increase in their total amount. Therefore, we hypothesised that the heating treatment applied did not significantly increase the

total amount of melanoidins in biscuits, but led to differences in the chemical structure of these compounds.

In line with this idea, significant differences were observed in the absorbance parameters of the isolated melanoidins (Table 1). These parameters are considered non-specific indicators of the extent of the Maillard reaction: K_{294} is associated with the presence of early MRP, while the browning can be followed by the increase of K_{420} (Delgado-Andrade et al., 2009; Pastoriza & Rufián-Henares, 2014). According to this result, although no significant differences were found in the melanoidin amount measured by the gravimetric method, the extent of MR in HT-BM was higher than in LT-BM.

3.2 Composition and antioxidant activity of extracted melanoidins

The isolated biscuit melanoidins were chemically analysed, and the results are listed in Table 1. Both samples presented a high proportion of proteins (more than 50%) and a moderate content of carbohydrates (lower than 40%). This result compares well with that reported by Pastoriza and Rufián-Henares (2014), who indicated that melanoidins extracted from cereal foods (such as cereals, bread and biscuits) had a high protein content (around 50%). Moreover, the results suggest that LT-BM had a higher proportion of proteins than HT-BM (57.0±3.6 and 52.0±2.0 % respectively) ($p \leq 0.05$). Nonetheless, HT-BM showed a higher proportion of phenolic groups than LT-BM (19.5±2.6% and 7.8±0.3% respectively) ($p \leq 0.05$). It is well known that MR encompasses a network of several reactions between reducing sugars and compounds with a free amino group, mainly proteins (Delgado-Andrade et al., 2009). Moreover, some authors have reported that phenolic compounds also play an important role in melanoidin formation as they may remain linked to their structure (Nunes & Coimbra, 2010). Our results seem to indicate that at low temperature, biscuit melanoidins present a more "classical" structure with a high proportion of proteins and carbohydrates, but when the melanoidins develop at higher temperatures phenolic compounds undergo MR and become an important part of the melanoidinic structure. Consistent with this observation, Shaheen et al. (2021) reported that melanoidins are multi-component polymers with similar molecular weight, but different binding preferences. According to these authors, during the development of MR, the heat treatment enhances the incorporation of phenolics into the protein-based melanoidin skeleton, increasing the total carbon content and the aromatic character of melanoidins. This might be because of the different reaction pathways that can be taken in real food systems where different compounds may be involved in MR. However, this hypothesis is only of preliminary nature and future work should further analyse it.

According to the results displayed in Table 1, the antioxidant capacity of HT-BM was significantly higher than that of LT-BM according to ABTS (274.96±21.61 vs 78.38±26.57 mmol Trolox/g); DPPH (7.69±3.10 vs 0 mmol Trolox/g) and FRAP (0.14±0.01 vs 0.03±0.001 mmol Trolox/g) ($p \leq 0.05$). Therefore, the high antioxidant capacity found in HT-BM could be associated with the presence of phenolic compounds in the melanoidin structure.

The presence of phenolic compounds in the melanoidins may have a beneficial effect on the digestive tract. Our previous studies indicated that during the digestion process of HT-biscuits, antioxidants were partially released, whereas a high proportion was available after fermentation by colonic bacteria (Patrignani et al., 2019). According to the current results this could account for the high proportion of phenolic compounds in HT-BM that the gut microbiota is able to release (Pérez-Burillo et al., 2020). Moreover, the presence of phenolic structures available for absorption through the epithelial wall could also explain the increase in the serum antioxidant capacity and the reduction of its oxidation levels found after HT-BM consumption (Patrignani et al., 2016).

3.3 DSC analysis of melanoidins

DSC analysis is a reliable methodology to study the thermal behaviour of food components, and the thermal transitions of melanoidins have been associated with the development of MR (Patrignani & González-Forte, 2021). In the present work, melanoidins were placed in hermetically sealed steel pans, and their thermograms were analysed (Fig. 1). While LT-BM presented an exothermal peak at 166±3.9°C followed by an endothermal peak at 196±5.1°C, HT-BM only presented one endothermal peak at 197±1.6°C. The two peaks have been previously observed in model systems by Kaspchak et al. (2022) and Manzocco et al. (1999). According to these authors, the exothermic transition can be attributed to the development of MR in the conditions of the DSC assay. Broyart et al. (1998) indicated that the critical temperature at which the darkening phase begins in biscuits is in the region of 105-115 °C, very close to the low temperature condition selected in our study (100 ± 10 °C). Therefore, a limited development of MR was observed despite the long cooking time (80 min). In contrast, the high temperature condition (150 ± 10 °C) was considerably higher than the critical temperature, and the development of a non-enzymatic reaction was favoured. Thus, the absence of exothermal transitions in melanoidins extracted from HT-BM indicates that the thermal treatment at 150°C induced a complete development of MR structures during the cooking process, which can also explain the high value of K_{420} found in these samples (Table 1).

The second endothermic peak is probably associated with the thermal degradation of proteinic arrangements in the melanoidin skeleton (Patrignani & González-Forte, 2021). Results showed that the enthalpy value (ΔH_{p2}) of this peak was significantly lower in HT-BM than in LT-BM (119.3±21.9 and 292.1±75.4 respectively) ($p \leq 0.05$). In line with this result, Table 1 shows that LT-BM contained a higher proportion of protein structure than HT-BM. Similar results were found by Manzocco et al. (1999) in glucose–glycine model systems, where the prolonged heating reduced the ΔH values and induced the development of brown final MRP. Therefore, in agreement with previous results, it could be concluded that the thermal treatment at 150°C enhanced the development of MR final products with a low proportion of proteins, while in biscuits prepared at 100°C the extent of MR is limited by the temperature used, and the melanoidin structure formed has a higher proportion of proteinic arrangements.

3.4 XRD and ZP of melanoidins

Broad diffraction peaks centred at 20° (2θ) were found in the melanoidins analysed. This result is in good agreement with Cai et al. (2022), who indicated that the crystallinity of the melanoidins in crystal malt was formed at 19° (2θ). Moreover, as displayed in Fig. 2a, as the thermal treatment increased, the crystallinity also increased (HT-BM presented a 30% increase in crystal structure compared to LT-BM). Although information about the XRD of melanoidins is scarce, the current results seem to indicate that during the development of MR in biscuits, the thermal treatment induces the formation of a stable crystal structure.

The *in vivo* analysis indicated that the food efficiency of the diet with HT-BM was significantly lower than that of the diet with LT-BM, and its intake was associated with a lower body weight (Patrignani et al., 2016). It is well known that the crystallinity of macromolecules, such as starch, is a key factor in their digestive rate, as densely-packed crystalline regions are more resistant to enzyme hydrolysis than the amorphous regions (Donato-Capel et al., 2014). Although the association between the crystallinity and the digestibility of melanoidins has not been studied yet, our results seem to point out that the melanoidin structure developed at high temperature during the cooking process could be more resistant to enzyme digestion, thus resulting in a reduced food efficiency of biscuits. Besides, melanoidins that escape from enzymatic hydrolysis could probably reach the large intestine to undergo fermentation (Patrignani et al., 2019). It should also be considered that according to Fogliano and Morales (2011),

melanoidins in biscuits are homogeneously distributed. Therefore, their presence may have a strong impact on the entire product and could also limit the absorption of other food components (Alves et al., 2021).

Melanoidins are considered to be negatively charged compounds, and this characteristic has been used to explain their ability to bind metallic ions (Morales et al., 2012). In the present paper, the ZP of biscuit melanoidins was determined, and the results are shown in Fig. 2b. The magnitude of ZP was significantly higher in HT-BM than in LT-BM (-36.8±0.6 and -16.8±0.1 respectively). Cai et al. (2022) reported similar values of ZP in melanoidins extracted from crystal malt and explained that high molecular-weight particles of the melanoidins had multiple charges on their surface. Moreover, Bekedam et al. (2008) found that the presence of subunits derived from chlorogenic acids correlated with the increasing ionic charge of coffee melanoidins. Our current results also indicate that the negative charge of melanoidins correlates well with their phenolic content (Table 1).

According to Wang et al. (2022) the ZP plays an important role in the zinc chelating capacity, as zinc ions can interact with the negative charges of some groups such as carboxyl groups ($-COO^-$) (Sun et al., 2021). Because of their health implications, the Zn chelating capacity of melanoidins has been extensively studied and associated with the antihypertensive capacity of MRP as the angiotensin-I converting enzyme (which regulates blood pressure) is Zn-dependent (Rufián-Henares & Morales, 2007). According to the present results, it could be hypothesised that the HT-BM presented a higher Zn chelating capacity, which would be in line with results from Wen et al. (2005), who indicated that the heating treatment in coffee brews induced the development of MRP with chelating capacity. However, these studies were performed *in vitro,* and the results from *in vivo* trials show an opposite trend. According to our results, the animals that were fed with a diet with HT-BM showed the same mineral absorption as the control group, whereas rats fed with LT-BM did show a reduction in their mineral absorption and the lowest values of Zn absorption (Patrignani et al., 2016). Moreover, Delgado-Andrade et al. (2016) also reported that the intake of bread crust with a high amount of MRP in rats did not produce any significant impact on the copper or zinc balances. Therefore, there is probably another effect that counteracts the chelating capacity of HT-BM during the *in vivo* digestion process.

3.5 FT-IR analysis of melanoidins

Fig. 3 shows the FT-IR spectra of LT-BM and HT-BM formed during the heating treatment of biscuits. For HT and LT-BM, the low contribution of the CH groups was reflected by the peak at 2927 cm^{-1} (Kang, 2016), and both this band and the one at 2855 cm^{-1} increased for HT-BM, which indicates a structure modification when MR takes place at high temperatures.

The region from 1800 to 800 cm^{-1} is often useful for the analysis of proteinaceous material that associates with non-enzymatic browning products. For HT-BM, a shoulder appeared at 1520 cm^{-1}, suggesting the presence of a Schiff base (C=N double bond, intermediate products) in the final structure of melanoidins developed at high temperatures (Khadidja et al., 2017). Also, the absorption band at 1743 cm^{-1} showed a significant increase for HT-BM, which, in accordance with Ravindran et al. (2018), could represent ester bonds or carboxylic linkages (C=O stretching vibration) from Amadori products. Furthermore, the peak at 1077 cm^{-1} showed an increase in intensity, in good line with the temperature rise, suggesting the presence of furanose rings in the melanoidin structure (Mutaillifu et al., 2020; Patrignani & González-Forte, 2021). Therefore, it could be inferred that although HT-BM underwent a strong heating treatment (as determined by the DSC analysis), some characteristic compounds from intermediate stages of MR remained in their structure, such as Amadori compounds and Schiff base (Delgado-Andrade et al., 2009).

The region between 1650 and 1540 cm^{-1} is related to amide I and amide II structures, including C=O stretching (amide I), C-N stretching and N-H deformation (amide II) (Liu et al., 2014). In this study, protein-based bonds of nC=O amide I (1635 cm^{-1}) were observed for HT-BM and LT-BM (Silbir & Goksungur, 2019). Also, the absorption band around 1300 cm^{-1} can be attributed to amide III structures, including C=O stretching, C-N stretching and N-H deformation. Changes of the amide III band at 1339 cm^{-1} in Fig. 3 show that hydroxyl and amino groups were consumed during the heating process. Moreover, the band at 1400 cm^{-1}, associated with the COO^- symmetric stretching, appeared in both HT-BM and LT-BM, although it was more intense in the first one. This band has been associated with the Zn chelation capacity, and this result supports the idea that melanoidins may act as an organic ligand, as previously explained (Sun et al., 2021; Wang et al., 2022).

Phenolic compounds are one of the most studied categories of chemical species because of their antioxidant capacity and their benefit for human health. In this study, the phenolic C–O stretching appeared more clearly at 1205 cm^{-1} for HT-BM. This stretching is due to the C–O of pyran, typical of flavonoid C-rings (Wongsa et al., 2022). This result could be related to the higher phenolic group content and antioxidant activity found in HT-BM (Table 1). Previous authors have indicated that phenolic groups could be combined with melanoidins and may be responsible for their antioxidant capacity (Pérez-Burillo et al., 2020); this hypothesis has been also explored in the present work (see Section 3.2). Nonetheless, according to our present results, the antioxidant capacity may be explained not only by these components, but by a combined action of the many different functional groups present in melanoidins. Therefore, future research should confirm the presence and reveal the structure of the phenolics attached to melanoidins, as well as other functional groups, through more specific methodologies such as mass spectrometry. Besides, the stability and the kinetic parameters associated to the structure development of melanoidins should also be studied at different time/temperatures conditions.

Finally, the region between 1180 and 953 cm^{-1} is generally related to polysaccharide C-O-C bond vibrational modes, such as C-C and C-O stretching, and C-H bending (Iadecola et al., 2022). As shown in Fig. 3, there was an increase of the absorption band at 1148 cm^{-1} for HT-BM, which may be associated with the formation of a melanoidin skeleton with carbohydrate side chains. Also, a decrease of around 1025 cm^{-1} was observed for HT-BM, probably due to a process of glycation and the participation in forming Maillard components when the heating rate increased (Khadidja et al., 2017).

4. Conclusions

Biscuit melanoidins are complex macromolecules with several health effects. The different heating treatments applied to the biscuits (at 100°C or 150°C) did not have a significant effect on the total amount of melanoidins in these bakery products, but led to differences in their chemical structure. HT-BM presented higher values of K_{420} and K_{294}, and showed a complete development of MR, had a higher proportion of phenolic compounds in their structure, a higher negative net charge and a more crystal structure than LT-BM. These characteristics were positively associated with the antioxidant capacity, the prebiotic effect and the lower food efficiency that these compounds presented in an *in vivo* trial. FTIR showed that the melanoidin structure had a skeleton with carbohydrate side chains, furanose rings, phenolic compounds and some characteristic compounds from intermediate stages of Maillard reaction. Although there is still much to understand about biscuit melanoidin structure, this first approach helps to reveal their complex arrangement. Future studies should evaluate more heating conditions to fully understand the development of Maillard reaction in biscuits.

CRediT authorship contribution statement

M. P.: Conceptualization; Formal analysis; Investigation; Methodology; Writing - original draft. L. G-F: Formal analysis; Methodology; Writing-review & editing. J.A. R-H; P.A.C: Supervision; Data curation; Formal analysis; 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 at the online version of this manuscript

References

Alves, G., Lobo, L.A., Domingues, R.M.C.P., Monteiro, M., & Perrone, D. (2021). Bioaccessibility and gut metabolism of free and melanoidin-bound phenolic compounds from coffee and bread. *Frontiers in Nutrition, 8,* Article 708928. https://doi.org/10.3389/fnut.2021.708928

Antonietti, S., Silva, A. M., Simões, C., Almeida, D., Félix, L. M., Papetti, A., & Nunes, F. M. (2022). Chemical Composition and Potential Biological Activity of Melanoidins from Instant Soluble Coffee and Instant Soluble Barley: A Comparative Study. *Frontiers in Nutrition*, *9*, Article 34. https://doi.org/10.3389/fnut.2022.825584.

Bekedam, E. K., Schols, H. A., Van Boekel, M. A., & Smit, G. (2008). Incorporation of chlorogenic acids in coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, *56*, 2055-2063. https://doi.org/10.1021/jf073157k.

Cai, G., Li, C., Cao, Y., Chen, X., Wu, D., Li, X., ... & Lu, J. (2022). Structure and functional characterization of melanoidins from crystal malt. *European Food Research and Technology*, 1-11. https://doi.org/10.1007/s00217-022-04186-6.

Delgado-Andrade, C., Roncero-Ramos, I., Haro, A., Pastoriza, S., & Navarro, M.P. (2016). Do bread-crust-derived Maillard reaction products affect the retention and tissue distribution of trace elements? *European Journal of Nutrition, 55,* 1225-1233. https://doi.org/10.1007/s00394-015-0935-9.

Delgado-Andrade, C., Rufián-Henares, J.A., & Morales, F.J. (2009). *Assessing the Generation and Bioactivity of Neo-Formed Compounds in Thermally Treated Foods* (1st ed.). Atrio SL.

Díaz-Morales, N., Cavia-Saiz, M., Rivero-Perez, M. D., Gómez, I., Salazar-Mardones, G., Jaime, I., ... & Muñiz, P. (2023). Bread melanoidins as potential new sustainable bakery ingredients: a study using fat and fat-free bakery food models. *Food & Function*, *14*, 1785-1794. https://doi.org/10.1039/D2FO03909E.

Donato-Capel, L., Garcia-Rodenas, C. L., Pouteau, E., Lehmann, U., Srichuwong, S., Erkner, A., ... & Sagalowicz, L. (2014). Technological means to modulate food digestion and physiological response. In M. Boland, M. Golding & H. Singh (Eds.), *Food structures, digestion and health* (pp. 389-422). Elsevier Academic Press.

Fogliano, V., & Morales, F. J. (2011). Estimation of dietary intake of melanoidins from coffee and bread. *Food & Function, 2,* 117-123. https://doi.org/10.1039/C0FO00156B

Iadecola, R., Ciccoritti, R., Ceccantoni, B., Bellincontro, A., & Amoriello, T. (2022). Optimization of Phenolic Compound Extraction from Brewers' Spent Grain Using Ultrasound Technologies Coupled with Response Surface Methodology. *Sustainability*, *14,* Article 3309. https://doi.org/10.3390/su14063309

Kang, O.J. (2016). Evaluation of melanoidins formed from black garlic after different thermal processing steps. *Preventive Nutrition and Food Science, 21,*398-405. https://doi.org/10.3746/pnf.2016.21.4.398

Kaspchak, E., Toci, A.T., Menezes, L.R.A., Igarashi-Mafra, L., & Mafra, M.R. (2022). Effect of phytic acid, tannic acid and saponin on asparagine-glucose Maillard reaction. *Food Chemistry*, *394*, Article 133518. https://doi.org/10.1016/j.foodchem.2022.133518.

Khadidja, L., Asma, C., Mahmoud, B. & Meriem, E. (2017). Alginate/gelatin crosslinked system through Maillard reaction: preparation, characterization and biological properties. *Polymer Bulletin*, *74*, 4899–4919. https://doi.org/10.1007/s00289-017-1997-z

Kurniawan, M. F., Andarwulan, N., Wulandari, N., & Rafi, M. (2017). Metabolomic approach for understanding phenolic compounds and melanoidin roles on antioxidant activity of Indonesia robusta and arabica coffee extracts. *Food Science and Biotechnology, 26*, 1475-1480. https://doi.org/10.1007/s10068-017-0228-6.

Liu, Q., Kong, B., Han, J., Sun, C., & Li, P. (2014). Structure and antioxidant activity of whey protein isolate conjugated with glucose via the Maillard reaction under dry-heating conditions. *Food Structure*, *1,* 145-154. https://doi.org/10.1016/j.foostr.2013.11.004

Lopes, G. R., Ferreira, A. S., Pinto, M., Passos, C. P., Coelho, E., Rodrigues, C., ... & Coimbra, M. A. (2016). Carbohydrate content, dietary fibre and melanoidins: Composition of espresso from single-dose coffee capsules. *Food Research International, 89,* 989-996. http://dx.doi.org/10.1016/j.foodres.2016.01.018.

Manzocco, L., Nicoli, M.C., & Maltini, E. (1999). DSC analysis of Maillard browning and procedural effects. *Journal of Food Processing and Preservation*, *23,* 317-328. https://doi.org/10.1111/j.1745-4549.1999.tb00388.x

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

Morales, F.J., Somoza, V., & Fogliano, V. (2012). Physiological relevance of dietary melanoidins. *Amino Acids*, *42*, 1097-1109. https://doi.org/10.1007/s00726-010-0774-1.

Mutaillifu, P., Bobakulov, K., Abuduwaili, A., Huojiaaihemaiti, H., Nuerxiati, R., Aisa, H.A., & Yili, A. (2020). Structural characterization and antioxidant activities of a water-soluble polysaccharide isolated from Glycyrrhiza glabra. *International Journal of Biological Macromolecules*, *144*, 751-759. https://doi.org/10.1016/j.ijbiomac.2019.11.245.

Nie, C., Li, Y., Qian, H., Ying, H., & Wang, L. (2022). Advanced glycation end products in food and their effects on intestinal tract. *Critical Reviews in Food Science and Nutrition*, *62*, 3103-3115. https://doi.org/10.1080/10408398.2020.1863904.

Nunes, F. M., & Coimbra, M. A. (2010). Role of hydroxycinnamates in coffee melanoidin formation. *Phytochemistry Reviews, 9,* 171-185. https://doi.org/10.1007/s11101-009-9151-7.

Nunes, F. M., Del Castillo, M. D., & Carbonero, F. (2022). Food Melanoidins: Chemistry and Nutrition. *Frontiers in Nutrition*, *9*, Article 881690. https://doi.org/10.3389/fnut.2022.881690

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., & González-Forte, L.S. (2021). Characterisation of melanoidins derived from Brewers' spent grain: new insights into their structure and antioxidant activity. *International Journal of Food Science & Technology, 56,* 384-391. https://doi.org/10.1111/ijfs.14653.

Patrignani, M., Rinaldi, G.J., & Lupano, C.E. (2016). *In vivo* effects of Maillard reaction products derived from biscuits. *Food Chemistry*, *196*, 204-210. https://doi.org/10.1016/j.foodchem.2015.09.038

Patrignani, M., Rinaldi, G.J., Rufián-Henares, J.Á., & 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.

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

Rajakaruna, S., Pérez-Burillo, S., Kramer, D. L., Rufián-Henares, J.Á., & Paliy, O. (2022). Dietary Melanoidins from Biscuits and Bread Crust Alter the Structure and Short-Chain Fatty Acid Production of Human Gut Microbiota. *Microorganisms*, *10,* Article 1268. https://doi.org/10.3390/microorganisms10071268.

Ravindran, R., Jaiswal, S., Abu-Ghannam, N., & Jaiswal, A.K. (2018). A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresource Technology*, *248*, 272-279. https://doi.org/10.1016/j.biortech.2017.06.039.

Rufián-Henares, J.A., & Morales, F.J. (2007). Functional properties of melanoidins: *In vitro* antioxidant, antimicrobial and antihypertensive activities. *Food Research International*, *40,* 995-1002. https://doi.org/10.1016/j.foodres.2007.05.002

Shaheen, S., Shorbagi, M., Lorenzo, J.M., & Farag, M. A. (2021). Dissecting dietary melanoidins: formation mechanisms, gut interactions and functional properties. *Critical Reviews in Food Science and Nutrition, 62*, 8954-8971. https://doi.org/10.1080/10408398.2021.1937509.

Silbir, S. & Goksungur, Y. (2019). Natural red pigment production by *Monascus Purpureus* in submerged fermentation systems using a food industry waste: Brewer's spent grain. *Foods*, *8,* Article 161. https://doi.org/10.3390/foods8050161.

Snelson, M., & Coughlan, M. T. (2019). Dietary advanced glycation end products: digestion, metabolism and modulation of gut microbial ecology. *Nutrients*, *11*, Article 215. https://doi.org/10.3390/nu11020215.

Sun, R., Liu, X., Yu, Y., Miao, J., Leng, K., & Gao, H. (2021). Preparation process optimization, structural characterization and in vitro digestion stability analysis of Antarctic krill (*Euphausia Superba*) peptides-zinc chelate. *Food Chemistry*, *340*, Article 128056. https://doi.org/10.1016/j.foodchem.2020.128056.

Tian, Z., Chen, S., Shi, Y., Wang, P., Wu, Y., & Li, G. (2023). Dietary advanced glycation end products (dAGEs): an insight between modern diet and health. *Food Chemistry, 415,* Article 135735. https://doi.org/10.1016/j.foodchem.2023.135735

Wang, B., Xiao, S., Chen, X., & Wang, J. (2022). Structural characterisation, gastrointestinal digestion stability and transepithelial transport study of casein peptide–zinc chelate. *International Journal of Food Science & Technology, 57*, 2770-2778. https://doi.org/10.1111/ijfs.15644.

Wen, X., Enokizo, A., Hattori, H., Kobayashi, S., Murata, M., & Homma, S. (2005). Effect of roasting on properties of the zinc-chelating substance in coffee brews. *Journal of Agricultural and Food Chemistry*, *53*, 2684-2689. https://doi.org/10.1021/jf048304i.

Wongsa, P., Phatikulrungsun, P., & Prathumthong, S. (2022). FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Scientific Reports*, *12,* Article 6631. https://doi.org/10.1038/s41598-022-10669-z

Xiang, S., Zou, H., Liu, Y., & Ruan, R. (2020). Effects of microwave heating on the protein structure, digestion properties and Maillard products of gluten. *Journal of Food Science and Technology*, *57,* 2139-2149. https://doi.org/10.1007/s13197-020-04249-0.

FIGURE CAPTIONS

Fig. 1. DSC curves of melanoidins isolated from biscuits prepared at high temperature (150°C) and low temperature (100°C) (HT-BM and LT-BM respectively).

Fig. 2. a) X-ray diffraction patterns and **b)** Zeta potential (ZP) of melanoidins isolated from biscuits prepared at high temperature (150°C) and low temperature (100°C) (HT-BM and LT-BM respectively).

Fig. 3. FT-IR spectra of melanoidins isolated from biscuits prepared at high temperature (HT-BM) and low temperature (LT-BM) in the 3800–650 cm^{-1} region.

Table 1. Chemical characterisation, absorbance parameters ($\mathbf{K_{294}}$ and $\mathbf{K_{420}}$) and antioxidant capacity (expressed as Trolox equivalent antioxidant capacity (TEAC) according to ABTS, DPPH and FRAP techniques) determined in the samples

	HT-BM*	LT-BM*
Content (g/100g)	7.6 ± 0.3[a]	6.8 ± 0.3[a]
$\mathbf{K_{294}}$ (L/g.cm)	14.0 ± 0.30[a]	2.50 ± 0.04[b]
$\mathbf{K_{420}}$ (L/g.cm)	3.75± 0.30[a]	1.5 ± 0.02[b]
Chemical characterisation		
Proteins (%)	52.0 ± 2.0[b]	57.5 ± 3.6[a]
Carbohydrates (%)	28.4 ± 2.7[a]	34.6 ± 2.0[a]
Phenolic groups (%)	19.5 ± 2.6[a]	7.8 ± 0.3[b]
Antioxidant activity		
$TEAC_{ABTS}$ (mmol Trolox/g)	274.96 ± 21.61[a]	78.38 ± 26.57[b]
$TEAC_{DPPH}$ (mmol Trolox/g)	7.69 ± 3.10[a]	0[b]
$TEAC_{FRAP}$ (mmol Trolox/g)	0.14 ± 0.010[a]	0.03 ± 0.001[b]

Results are expressed as mean ± standard deviation. Values in the same line followed by different superscript letters are significantly different ($p \leq 0.05$).

* HT-BM and LT-BM for high temperature (150°C/25min) and low temperature (100°C/80min) biscuit melanoidin respectively.

CRediT authorship contribution statement

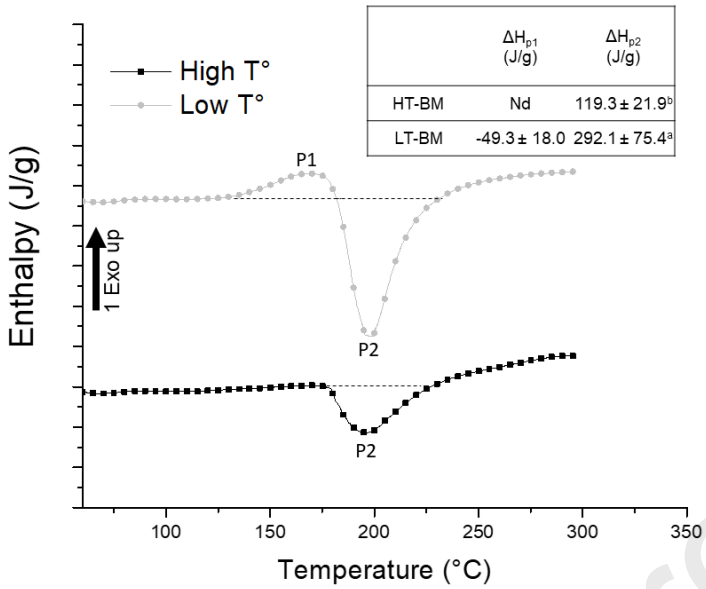
M. P.: Conceptualization; Formal analysis; Investigation; Methodology; Writing - original draft. L. G-F: Formal analysis; Methodology; Writing-review & editing. J.A. R-H; P.A.C: Supervision; Data curation; Formal analysis; Writing-review & editing.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

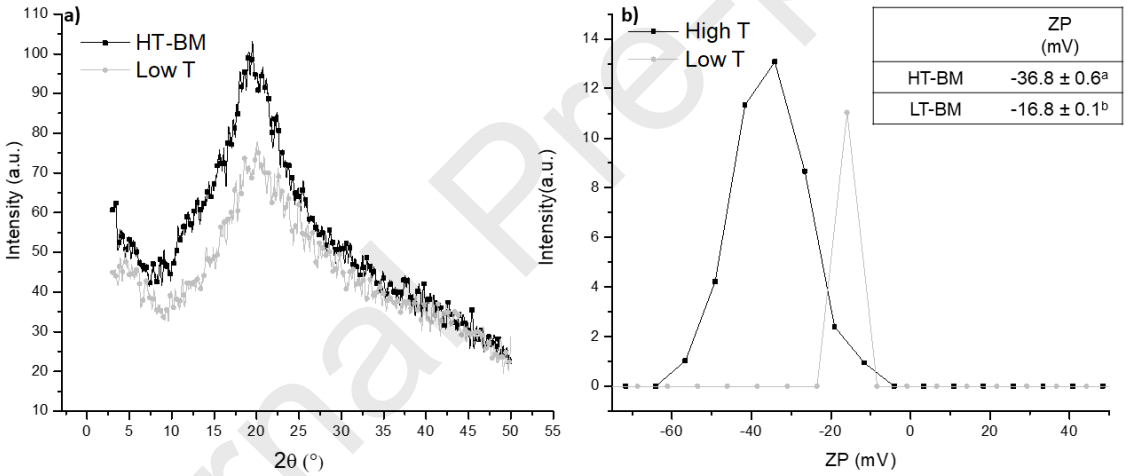
Fig. 1.



	ΔH_{p1} (J/g)	ΔH_{p2} (J/g)
HT-BM	Nd	119.3 ± 21.9[b]
LT-BM	-49.3 ± 18.0	292.1 ± 75.4[a]

505

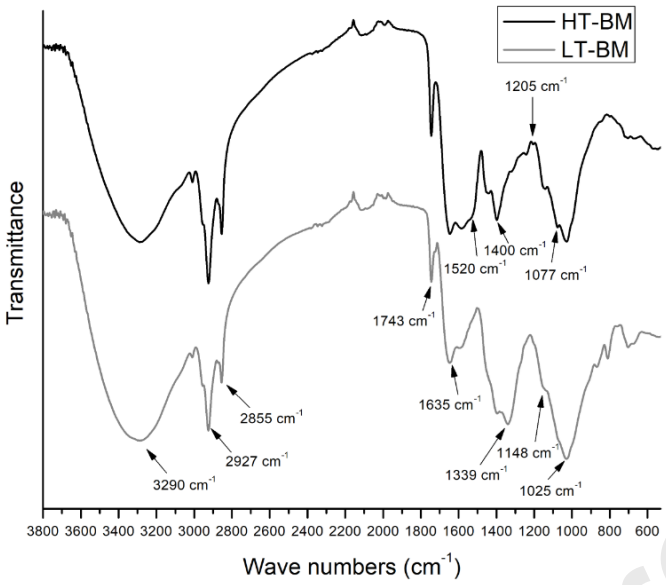
Fig. 2.

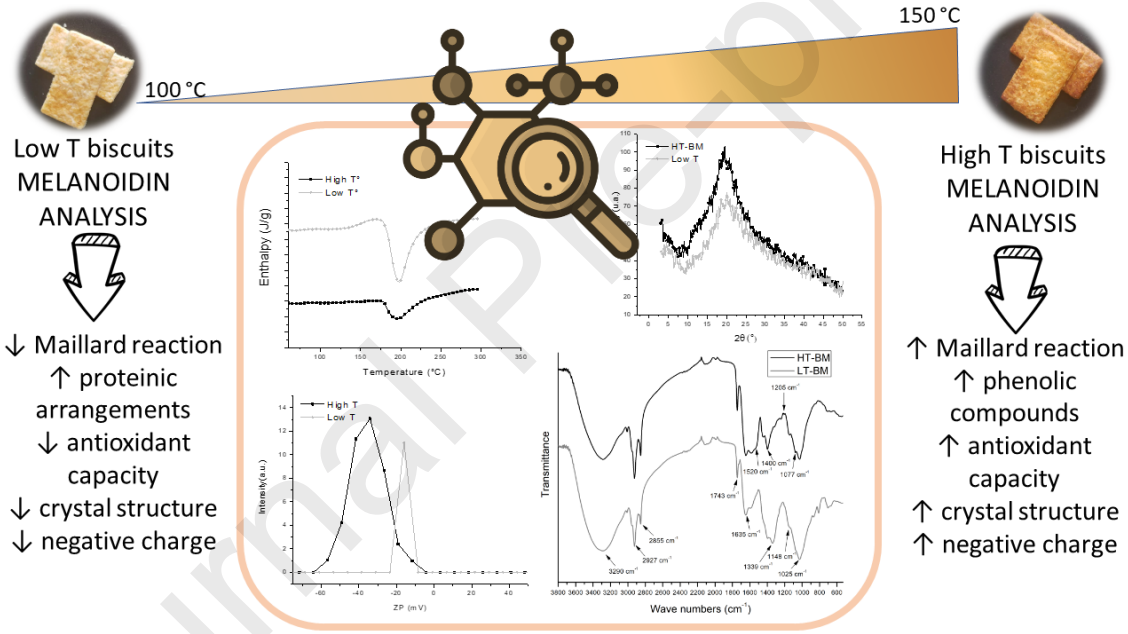


	ZP (mV)
HT-BM	-36.8 ± 0.6[a]
LT-BM	-16.8 ± 0.1[b]

506

Fig. 3.





Highlights

- Biscuit melanoidin benefits were related to their structure, not to their amount
- The processing temperature increased the melanoidin phenolic content
- The crystal structure of melanoidins might determine their digestibility
- Melanoidin composition and negative charge depended on the thermal treatment