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REVIEW

Coffee melanoidins: structures, mechanisms of formation and potential health impacts

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During the roasting process, coffee bean components undergo structural changes leading to the formation of melanoidins, which are defined as high molecular weight nitrogenous and brown-colored compounds. As coffee brew is one of the main sources of melanoidins in the human diet, their health implications are of great interest. In fact, several biological activities, such as antioxidant, antimicrobial, anticariogenic, anti-inflammatory, antihypertensive, and antiglycative activities, have been attributed to coffee melanoidins. To understand the potential of coffee melanoidin health benefits, it is essential to know their chemical structures. The studies undertaken to date dealing with the structural characterization of coffee melanoidins have shown that polysaccharides, proteins, and chlorogenic acids are involved in coffee melanoidin formation. However, exact structures of coffee melanoidins and mechanisms involved in their formation are far to be elucidated. This paper systematizes the available information and provides a critical overview of the knowledge obtained so far about the structure of coffee melanoidins, mechanisms of their formation, and their potential health implications.

1. Introduction

The roasting of green coffee beans is an important step in coffee processing. The characteristic aroma, taste, and color of the coffee brew, prepared by hot water extraction from roasted and ground coffee beans, are to a great extent determined by the roasting process.[1–5] During this process, the chemical composition of the beans is changed due to the degradation and/or transformation of some of the compounds identified in green coffee beans. Green coffee beans are, on a dry matter basis, mainly composed by carbohydrates (59–61%), lipids (10–16%), proteins (10%), and chlorogenic acids (7–10%), containing lower amounts of minerals (4%), aliphatic acids (2%), caffeine (1–2%), trigonelline (1%), and free amino acids (<1%). Upon roasting, a decrease is observed in coffee bean carbohydrates (38–42%), proteins (8%), chlorogenic acids (3–4%), and free amino acids, whereas lipids (11–17%), minerals (5%), aliphatic acids (3%), caffeine (1–2%), and trigonelline (1%) keep their relative

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From left to right : Ana S. P. Moreira, Fernando M. Nunes, M. Rosário Domingues, Manuel A. Coimbra

Ana S. P. Moreira initiated in the present year her PhD project in the Department of Chemistry at the University of Aveiro (Portugal), under the scientific supervision of Dr M. Rosário Domingues and Prof. Manuel A. Coimbra. Her work is focused on the study of structural modifications induced by thermal and oxidative treatments in model oligosaccharides and polysaccharides isolated from coffee, using mass spectrometry as the main technique for structural analysis. She received her BA in Biochemistry in 2009 and her Master in Analytical Chemistry and Quality in 2011 at the University of Aveiro.

content.[1] Also, as a result of the transformations occurring during the coffee roasting, melanoidins are formed.[6]

Melanoidins are the final products of the Maillard reaction.[7–9] This non-enzymatic browning reaction encompasses a network of various reactions between reducing sugars and compounds with a free amino group forming a variety of products, which can be classified as early stage products, intermediate stage products, and last stage products, the melanoidins.[7] Melanoidins are generically defined as high molecular weight nitrogenous brown-colored compounds.[6,10] Scarce information is available about their chemical structures, although they are formed during the heat processing of a large range of food products beyond coffee, such as bread,[11] malt,[12] meat,[13] and tomato sauce.[14] Because melanoidins cannot be directly analyzed due to the uncertainty of their structures, they are usually quantified by difference, subtracting the total percentage of known compounds from 100 percent. Using this criterion, they were estimated to account for up to around 25% (w/w) of the dry weight of roasted coffee beans.[1,15]

In coffee brew, melanoidins were estimated to account for up to around 29% (w/w) of the dry matter, quantified by difference.[15] The melanoidin content in coffee brew (and in coffee brew fractions) has also been evaluated based on their contribution to the brown color of the brew using color dilution analysis,[16,17] or by measuring the absorption near 400 nm,[18–26] particularly at 405 nm.[20–26] The absorption spectrum from 200 to 700 nm of a coffee brew shows two absorption maxima, one at 280 nm and a second at 325 nm. The absorption maximum at 280 nm can be explained by the presence of the aromatic rings of proteins, caffeine, chlorogenic acids, and caffeic acid. The absorption maximum at 325 nm can be explained by the presence of chlorogenic acids and caffeic acid.[22] The melanoidin content in coffee brew has been evaluated by measuring the absorption at 405 nm, since this is a wavelength often chosen to measure the intensity of the brown color. To be able to compare absorption values at 405 nm of different coffee brews (and coffee brew fractions), Bekedam *et al.*[22] proposed to express the absorption (A) at this wavelength as $K_{\text{mix 405nm}}$, which is the specific extinction coefficient at 405 nm calculated using the law of Lambert–Beer, expressed as $A = K_{\text{mix}}$ ($\text{L g}^{-1}\text{ cm}^{-1}$) × concentration ($\text{g L}^{-1}$) × length of light path (cm). Comparing coffee brews prepared from roasted coffee beans with different degrees of roast (and their high molecular weight fractions), an increase of the $K_{\text{mix 405nm}}$ value with increasing the degree of roast was observed,[20] suggesting a direct relation between the melanoidin content and the degree of roast.

Coffee brew is considered one of the main sources of melanoidins in the human diet, since it is consumed by millions of people worldwide every day.[11] Several studies on coffee melanoidins have been performed since at least the 1960s.[27] These studies, focused primarily on melanoidins structural characterization, have more recently been extended to their biological activities and effects on human health. In this paper a critical overview is presented about what has been published to date about the structure of coffee melanoidins, mechanisms of their formation, and their potential health implications.

2. Coffee melanoidin structures

Despite all efforts, the chemical structure of coffee melanoidins remains largely unknown. On the basis of current knowledge, it can be stated that the structural elucidation of coffee melanoidins is hampered by the extreme complexity and diversity of their structures and the consequent difficulty in isolation of pure melanoidin fractions.

Different approaches have been employed for isolation and purification (if any) of coffee brew melanoidins.[6] In all approaches, coffee melanoidins are first isolated taking advantage of their high molecular weight. The high molecular weight material (HMWM) of coffee brews has been isolated by dialysis using membranes with a molecular weight cut-off (MWCO) of 2 kDa[28] and 12–14 kDa,[16] by diafiltration using a hollow fiber with a MWCO of 3 kDa,[20,22,23] and by stepwise tangential flow ultrafiltration using membranes with a MWCO of 100, 50, 10, and 3 kDa.[17] Also, the HMWM has often been isolated by ultrafiltration using a membrane with a MWCO of 10 kDa.[29–36] Uncommonly, the high molecular weight coffee brew fractions have been isolated by gel filtration chromatography, monitoring

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the elution profile by using the absorbance at 405 nm.[21,26] The diversity of separation techniques used, namely dialysis, diafiltration, and ultrafiltration using membranes with different MWCOs, shows that the different authors did not yet establish a consensus for the minimum molecular weight for melanoidins.

Because the HMWM of the coffee brew prepared from roasted beans comprises proteins and polysaccharides beyond melanoidins,[37–39] additional purification procedures are required for their separation. Over the last decade, different melanoidin populations have been purified from the HMWM of the coffee brew.[16,17,20,23,25] The purification of different melanoidin populations has been achieved exploiting different physico-chemical properties such as solubility, charge, metal chelating ability, and hydrophobicity. The HMWM has been fractionated into fractions with different polysaccharide composition using an ethanol fractionation procedure. The fractions most enriched in galactomannans precipitate at an ethanol concentration of 40–50% and the fractions most enriched in arabinogalactans precipitate at an ethanol concentration of 75–80% (% by volume or weight). The fractions that remain soluble at an ethanol concentration of 75–80% present the lowest carbohydrate content.[16,22,37–39] Based on the anionic nature of coffee melanoidins,[19] anionic melanoidin fractions have been isolated performing anion exchange chromatography on the fractions obtained by ethanol fractionation,[16] or on the HMWM isolated directly from the coffee brew.[23,25] Also, due to the metal chelating capacity of coffee melanoidins,[40,41] a further fractionation of the anionic melanoidin fractions performed by copper affinity chromatography allows the isolation of anionic and chelating melanoidin fractions.[16] Also, some studies have shown that arabinogalactans in the form of arabinogalactan-proteins (AGPs) are present in green and roasted coffee beans as well as in green and roasted coffee brews, the latter including instant coffee.[23,42–46] Melanoidin fractions containing intact AGPs have been isolated from the HMWM of (roasted) coffee brews by precipitation with the AGP-specific β-glucosyl Yariv reagent.[20,23] In addition, hydrophobic melanoidin fractions have been isolated from the HMWM by gel permeation chromatography on Sephadex LH-20 followed by hydrophobic interaction chromatography.[17]

In respect to the physico-chemical properties of the melanoidins present in the HMWM of the coffee brew, new insights have been gained during the last decade using the purification procedures above described. It is now known that a fraction of coffee melanoidins present anionic character whereas other fractions do not present it or present it in a very low extent.[16,23] This heterogeneity is also extended to their metal chelating capacity, where about half of the anionic melanoidin fractions have chelating ability for immobilized copper ions.[16] Moreover, there are coffee melanoidin fractions that present hydrophobic character.[17]

It has been suggested that carbohydrates, amino acids, and phenolic compounds are components of coffee melanoidins.[27,47–49] Over the last decade, the chemical characterization of isolated and purified melanoidin fractions by a wide range of analyses, including analysis of sugars, glycosidic linkages, amino acids, nitrogen content, and phenolic groups content, has given increasing evidences that polysaccharides, proteins, and chlorogenic acids are involved in the formation of coffee melanoidins.[16,17,20,23,25] The following sections summarize what is known about the involvement of polysaccharides, proteins, and chlorogenic acids in the structures of coffee melanoidins.

2.1. The contribution of polysaccharides

Polysaccharides are the predominant carbohydrates present in green coffee beans, comprising about 50% of the dry weight of the beans. Galactomannans and type II arabinogalactans are their most abundant polysaccharides.[1,50] The galactomannans extracted with hot water from green coffee beans are composed by a main backbone of β-(1 → 4)-linked D-mannose residues, some of them substituted at *O*-6 by single residues of α-D-galactose or L-arabinose and at *O*-2 and/or *O*-3 by acetyl groups. Regarding to the acetylated mannose residues, there are single acetylated residues, di-acetylated residues and consecutively acetylated residues. Also, β-(1 → 4)-linked D-glucose residues are components of the mannan backbone.[51] Fig. 1a shows the main structural features of hot water extractable green coffee galactomannans. The arabinogalactans extracted with hot water from green coffee beans are composed by a main backbone of β-(1 → 3)-linked D-galactose residues, some of them substituted at *O*-6 with short chains of β-(1 → 6)-linked D-galactose residues. The galactose residues of these short chains are substituted with various combinations of α-L-arabinose, α-L-rhamnose, and β-D-glucuronic acid residues.[43] Almost all arabinogalactans in green coffee beans are covalently linked to proteins, so called arabinogalactan–proteins (AGPs).[42] Fig. 1b shows the main structural features of hot water extractable green coffee arabinogalactans (the polysaccharide moiety of AGPs).

During coffee roasting, arabinogalactans and galactomannans undergo several structural modifications. Based on sugar and

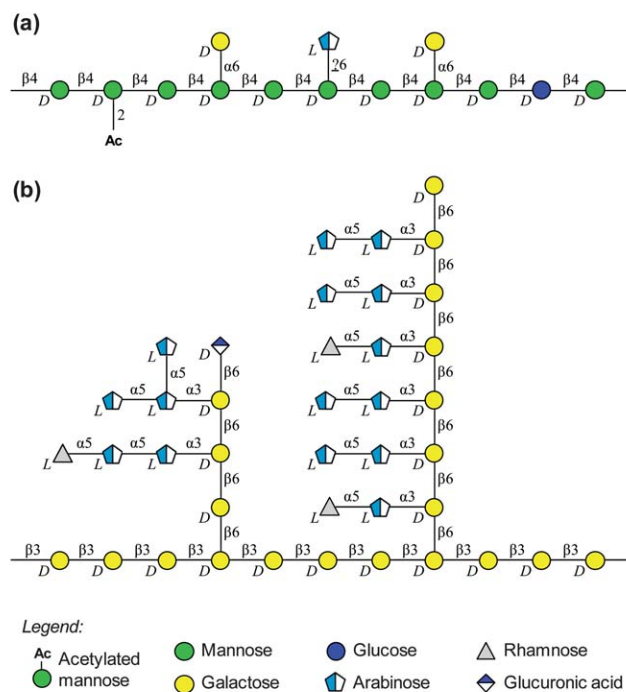

Fig. 1 Illustration of the main structural features of galactomannans (a) and arabinogalactans (b) isolated by hot water extraction of green coffee beans.

methylation analyses of the high molecular weight material (HMWM) isolated from green and roasted coffee brews, it has been stated that the roasting of green coffee beans promotes the decrease of the degree of polymerization as well as the debranching of these polysaccharides. In particular, arabinose, mainly present as side chains in the arabinogalactans, was found to be the sugar most sensitive to degradation during roasting.[37–39,52,53] However, dry thermal treatments on manno- and galactomanno- oligosaccharides, structurally related with coffee galactomannans, showed the occurrence of polymerization reactions.[54] This finding raised the hypothesis that the roasting of green coffee beans also promotes the polymerization of the galactomannans. Also, a detailed study on roasted coffee brew galactomannans showed that their reducing end is modified by the occurrence of caramelization, isomerization, oxidation, decarboxylation, and Maillard reactions.[55]

Melanoidin populations with different polysaccharide compositions have been isolated from the HMWM of coffee brews.[16,20,23,28] Based on the sugar composition of the fractions obtained from the HMWM of a roasted coffee brew by ethanol fractionation, Bekedam *et al.*[22] suggested the involvement of polysaccharides, especially arabinogalactans, in coffee melanoidin formation. The existence of polysaccharides covalently linked in melanoidins was proven later on by Nunes and Coimbra[16] by the observation of galactomannans and arabinogalactans in anionic fractions isolated from the ethanol fractions of roasted coffee brews by anion exchange chromatography, which contrasted with the negligible retention observed for the green coffee polysaccharides under the same chromatographic conditions.

Arabinogalactans can be incorporated in coffee melanoidin structures in the form of intact AGPs.[23] This was demonstrated by the addition of the Yariv reagent (a reagent that selectively precipitates AGPs) to the HMWM of a roasted coffee brew. Instead of the white color of the AGP fraction previously recovered from green coffee beans,[56] a brown colored precipitate was obtained. Based on the $K_{\mathrm{mix}\ 405\mathrm{nm}}$ values, it was shown that the AGP fraction accounts for approximately half of the melanoidins present in the HMWM of the coffee brew. However, as the amount of the galactose and arabinose of the AGP fraction obtained by precipitation with the Yariv reagent is smaller than that observed in the HMWM, it can be inferred that some of the green coffee bean AGPs are transformed during roasting by losing their protein moiety, as suggested by Bekedam *et al.*[23]

The studies carried out so far on coffee melanoidin polysaccharides showed that they are covalently-linked components of these high molecular weight brown structures. However, the types of linkages between the polysaccharides and the other components are not yet known.

2.2. The contribution of proteins

Proteins of green coffee beans, accounting for about 10% of bean dry weight, can be divided according to their solubility in water: 50% are water-soluble and 50% are water-insoluble proteins.[1] It is also known that coffee beans contain 11 *S*-type storage proteins,[57,58] representing almost 45% of total proteins.[59]

The roasting process leads to protein denaturation with degradation, the later inferred by the decrease in total amount of amino acids identified in coffee beans upon roasting. Among the amino acids that make up green coffee proteins, some of them, such as arginine, cysteine, lysine, and serine, showed a high decrease in their amount during roasting.[1,15,22,60] The changes in green coffee proteins promoted by roasting have also been followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).[37–39,61] The SDS-PAGE patterns obtained under nonreducing conditions of the HMWM isolated from green and roasted coffee brews are clearly distinct. While green coffees presented a major protein band at 58 kDa and a second one at 38 kDa, roasted coffees presented a defined band with ≤14 kDa and a diffuse band with >200 kDa,[37–39] the latter possibly due to the involvement of proteins in melanoidins formation.

Different melanoidin fractions have been isolated from the HMWM of coffee brews containing variable amounts of protein-like materials, quantified by amino acid analysis after acid hydrolysis.[16,17,22] Comparing different melanoidin fractions obtained from ethanol fractionation of the HMWM, it was noticed that the fraction that remains soluble at a high ethanol concentration (75–80%) has the highest protein content. However, the amino acid composition (in terms of amino acid relative abundances) is similar for all these melanoidin fractions. Alanine, aspartic acid/asparagine, glutamic acid/glutamine, and glycine are among the most abundant amino acids in all fractions, whereas histidine, lysine, methionine, and tyrosine are the least abundant. Arginine was not found.[16,22] The amino acid composition of these melanoidin fractions is also similar to those reported for roasted coffee beans and roasted coffee brews.[22] Moreover, various melanoidin fractions contain hydroxyproline,[16] an amino acid found in high amounts in AGPs isolated from green coffee beans and green coffee brews.[42,43] The presence of hydroxyproline in melanoidin fractions containing also arabinose and galactose residues is more evidence for the existence of AGPs in coffee melanoidin structures, as discussed earlier.

Amino acids account for the majority of the nitrogen present in the HMWM of the roasted coffee brew (>70%) and in the melanoidin fractions obtained from the HMWM by ethanol fractionation. Also, the amount of non-amino acid nitrogen is higher in the melanoidin fraction with highest ethanol solubility. This distinction between nitrogen from intact amino acids and non-amino acid nitrogen, also referred as non-protein nitrogen, has been achieved based on the amino acid/protein content determined by amino acid analysis after acid hydrolysis and the total nitrogen content.[22] However, the degraded/modified amino acids may account for the nitrogen of non-proteic origin determined using this approach. Effectively, Maillard reaction products derived from lysine, including N^{ε}-(fructosyl)lysine (FL, detected as furosine after acid hydrolysis), N^{ε}-(carboxymethyl) lysine (CML), and N^{ε}-(carboxyethyl)lysine (CEL) (Fig. 2), were identified in the HMWM and melanoidin fractions.[16] The identification of these compounds, although at very low amounts, suggests that amino acids modified during coffee roasting are also incorporated in melanoidin structures.

2.3. The contribution of chlorogenic acids

Phenolic compounds of green coffee beans are predominantly chlorogenic acids (CGAs), a family of esters formed between

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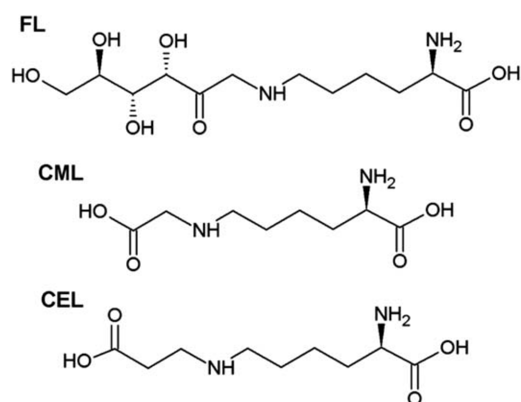

Fig. 2 Maillard reaction products identified in coffee melanoidin structures: N^{ε}-(fructosyl)lysine (FL), N^{ε}-(carboxymethyl)lysine (CML), and N^{ε}-(carboxyethyl)lysine (CEL).

quinic acid and *trans*-cinnamic acids, such as caffeic, *p*-coumaric, and ferulic acids.[1,62,63] More than 60 CGAs were already identified in green coffee beans,[64] being the most abundant 5-*O*-caffeoylquinic acid (5-CQA, Fig. 3).[65,66]

Total chlorogenic acid content found in green coffee beans account for up to 10% of their dry weight.[1] Upon roasting, the amount of hot water extractable chlorogenic acids decreases by 50% or more, depending on roasting intensity.[65] In respect to the products derived from the thermal degradation of CGAs during coffee roasting, it is already known that they are diverse and range from simple phenols to condensation products of high structural complexity, as recently reviewed by Nunes and Coimbra.[6] Briefly, the coffee roasting process promotes the isomerization of CGAs, as well as their hydrolysis yielding quinic acid (non-phenolic moiety) and various cinnamic acids (phenolic moieties).[63,67,68] Model studies on dry thermal treatment of 5-CQA, caffeic acid, and ferulic acid suggest the occurrence of decarboxylation of quinic and cinnamic acids during coffee roasting, yielding a range of simple phenols.[69–71] For caffeic acid, several condensation products were also identified.[71] Accordingly, bitter compounds present in coffee brews seem to be generated by oligomerization of a simple phenol (4-vinylcatechol) released from caffeic acid moieties upon roasting.[72] Furthermore, CGAs can be converted into chlorogenic acid lactones by the loss of a water molecule from the quinic acid moiety and formation of an intramolecular ester bond (Fig. 4).[66,67,73]

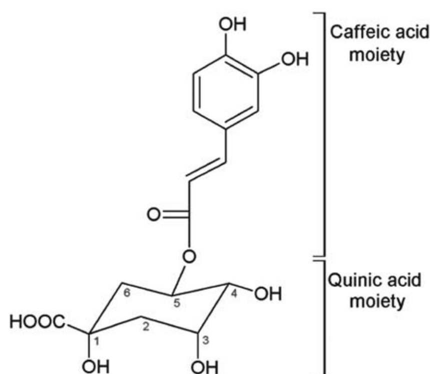

Fig. 3 Structure of 5-*O*-caffeoylquinic acid (5-CQA).

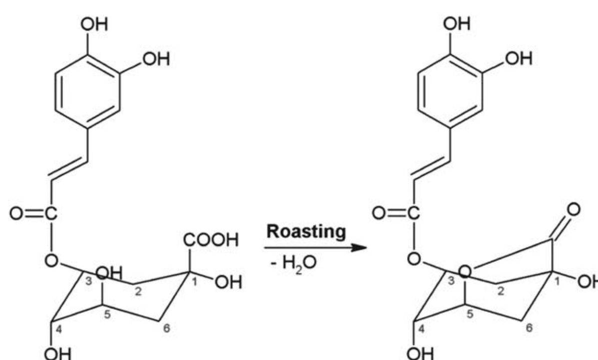

Fig. 4 Formation of a 1,5-γ-quinolactone from 3-*O*-caffeoylquinic acid (3-CQA) occurring during roasting as proposed by Farah *et al.*[73]

Various melanoidin fractions containing CGAs (or their derivatives) have been isolated from the HMWM of coffee brews.[16,20,25] The presence of CGAs in the HMWM and melanoidin fractions was suggested based on the quantification of phenolic compounds using the Folin–Ciocalteu reagent.[22,23] However, this colorimetric assay is not specific for phenolic compounds, but measures reducing compounds.[74] The incorporation of CGAs in coffee melanoidins was also suggested by the detection of phenols, such as guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol, after thermal degradation of the HMWM isolated from a coffee brew,[14] and also identified after thermal treatment of ferulic acid.[69] However, this approach does not allow evidencing the type of interaction (covalent or non-covalent) between CGAs and other components. The presence of covalently-linked CGAs (or their derivatives) in the HMWM and melanoidin fractions was demonstrated using the alkaline fusion method, known as an efficient method to release condensed phenolic structures.[16] To ensure the absence of non-covalently-linked CGAs, since non-covalently-linked CGAs were previously shown to occur in melanoidin fractions isolated from instant coffees,[29] the fractions subjected to alkaline fusion were previously submitted to a reversed phase high performance liquid chromatography after overnight incubation in 2 M NaCl. For both HMWM and melanoidin fractions, the most abundant of the monomeric phenolic compounds recovered after alkaline fusion was 3,4-dihydroxibenzoic acid. This compound was also the most abundant upon the alkaline fusion of ferulic and caffeic acid standards.[16] Alkaline fusion of a melanoidin fraction obtained from instant coffee by zinc precipitation gave similar monomeric phenolic compounds.[41] On the basis of these results, it can be stated that CGAs and/or their derivatives are incorporated in coffee melanoidin structures. The incorporation of 5-CQA, and caffeic, ferulic, and quinic acids was also evaluated in two subsequent studies using both the HMWM and the intermediate-MWM (IMWM) isolated from a coffee brew.[20,25] These studies showed the presence of ester linked caffeic, ferulic, and quinic acids, released after alkaline saponification. Also, these ester linkages were observed in the melanoidin fractions isolated from both HMWM and IMWM, with a more abundant presence of quinic acid than caffeic and ferulic acids. The presence of intact CGAs into the melanoidin structures, incorporated *via* caffeic acid moiety through mainly non-ester linkages, was inferred by the enzymatic treatment with chlorogenate esterase (EC 3.1.1.42).[25] In other studies, two-dimensional nuclear

magnetic resonance (2D NMR) analysis did not support the idea of intact caffeic or ferulic acid moieties integrated into the melanoidins of the high molecular weight coffee fraction.[17,28] The different results reported about the integration of intact cinnamic acid moieties into the coffee melanoidins point out the need of future studies designed to investigate this point.

Although there is currently strong evidence that CGA derivatives are components of coffee melanoidins, it is not yet known how they are linked within the melanoidin structure. Nunes and Coimbra[6] suggested that proteins can be a possible binding site for the CGA derivatives. This hypothesis was proposed based on studies demonstrating that the changes in green coffee protein profiles observed during roasting are similar to what are observed when oxidized CGAs are reacted with green coffee proteins in model systems.[61,75] Also, carbohydrates, particularly arabinose residues, seem to be a possible binding site for the CGA derivatives, as suggested by Bekedam *et al.*[25] This hypothesis was proposed based on studies demonstrating that the arabinose residues are quite susceptible to degradation during roasting,[46,53] and on a model study developed under simulated roasting conditions in which the reaction of epicatechin with sugar fragments is reported to occur.[76]

3. Formation mechanisms of coffee melanoidins

Although some structural features of coffee melanoidins have already been elucidated, the mechanisms involved in their formation are far to be completely understood.

Based on model studies (mostly in solution), three theories on melanoidin formation have been described.[6,10] One theory is that the melanoidins are formed by polymerization (*via* polycondensation reactions) of low molecular weight (LMW) Maillard reaction products, such as furans and pyrroles, formed in the advanced stages of the reaction.[77–79] Hofmann[80–82] has suggested that the melanoidins are derived from cross-linking of LMW Maillard reaction products to proteins *via* reactive side chains of amino acids such as lysine, arginine, and cysteine. The third theory is that the melanoidin skeleton is mainly built up of sugar degradation products, formed in the early stages of the Maillard reaction and polymerized through aldol-type condensation.[83–85]

Analysis of the volatiles released upon the thermal degradation of the HMWM isolated from a coffee brew revealed the presence of mostly furans (65%) followed by carbonyl compounds (16%).[14] The protein-bound 1,4-bis-(5-amino-5-carboxy-1-pentyl)pyrazinium radical cation (CROSSPY) was shown to be formed during roasting of coffee beans,[86] but also during heating of aqueous solutions of bovine serum albumin and glycolaldehyde used as model systems for the melanoidin formation.[87] These reports suggest that the three theories proposed for melanoidin formation may occur during coffee melanoidin formation. Also, the identification of the CROSSPY radical suggests that radical mechanisms may be involved in the formation of coffee melanoidins.

Fig. 5 is a simplistic illustration of coffee melanoidin formation, since the exact mechanisms involved in their formation remain unclear. This is an adaptation from the original produced by Nunes and Coimbra,[6] aiming to include the possible occurrence of galactomannan polymerization, as was reviewed here. In summary, polysaccharides, proteins, and chlorogenic acids (phenolic compounds) are known to be involved in coffee melanoidin formation. However, several questions remain open about their structures as the nature of the unknown material that can contribute up to 90% to the melanoidin weight, as well as the type of linkages between polysaccharides, proteins, and chlorogenic acids. Thus, the structural characterization of coffee melanoidins remains a topic of great research interest in the near future.

4. Potential health impacts of coffee melanoidins

As coffee brew is one of the main sources of melanoidins in the human diet,[11] biological activities of coffee melanoidins and their health implications are of great interest. As shown in Table 1 and described below, different biological activities have been attributed to coffee melanoidins. However, their physiological relevance is so far yet to be elucidated. First, studies on biological activities of coffee melanoidins have often been developed using the high molecular weight material (HMWM) isolated from coffee brews without subsequent purification, simply denominated as melanoidins. This approach is limited in the sense that the HMWM, as previously discussed, comprises other high molecular weight compounds, hampering a definitive conclusion about the active principle responsible for the biological activity. On the other hand, little is known about the metabolic transit and biotransformation of melanoidins (from coffee and other foods), as was previously reviewed.[88,89] Regarding coffee, the HMWM isolated from a coffee brew by ultrafiltration (MWCO 10 kDa) was digested *in vitro* by simulating gastrointestinal enzymatic digestion. The low molecular weight fraction recovered after digestion represented 14% of the HMWM, suggesting that the coffee melanoidins are largely resistant to digestion in the human gastrointestinal tract.[31] However, little else is known about the metabolic transit and biotransformation of coffee melanoidins. In fact, one question that remains unclear is whether the biological activities attributed to coffee melanoidins (mostly based on *in vitro* studies) have a significant impact on human health. This question is particularly relevant for those activities whose potential health effects rely on the presence of melanoidins in the bloodstream or their transportation through the blood to the organs, since melanoidins are high molecular weight compounds by definition and their ability to cross the intestinal epithelial barrier has not yet been reported.

The following sections are intended to present the studies performed to date on biological activities of coffee melanoidins, namely, their reported antioxidant activity and ability to inhibit matrix metalloproteases, the antimicrobial activity and ability to modulate the bacterial colon population, as well as the anti-cariogenic, anti-inflammatory, antihypertensive, and anti-glycative activities.

4.1. Antioxidant activity

Several studies have shown that coffee melanoidins (most referring to the HMWM isolated from coffee brews without subsequent purification) present *in vitro* antioxidant activity.[17,21,29–31] Based on the observation that low molecular weight compounds released from the HMWM of coffee brews after overnight incubation in 2 M NaCl showed higher antioxidant activity than

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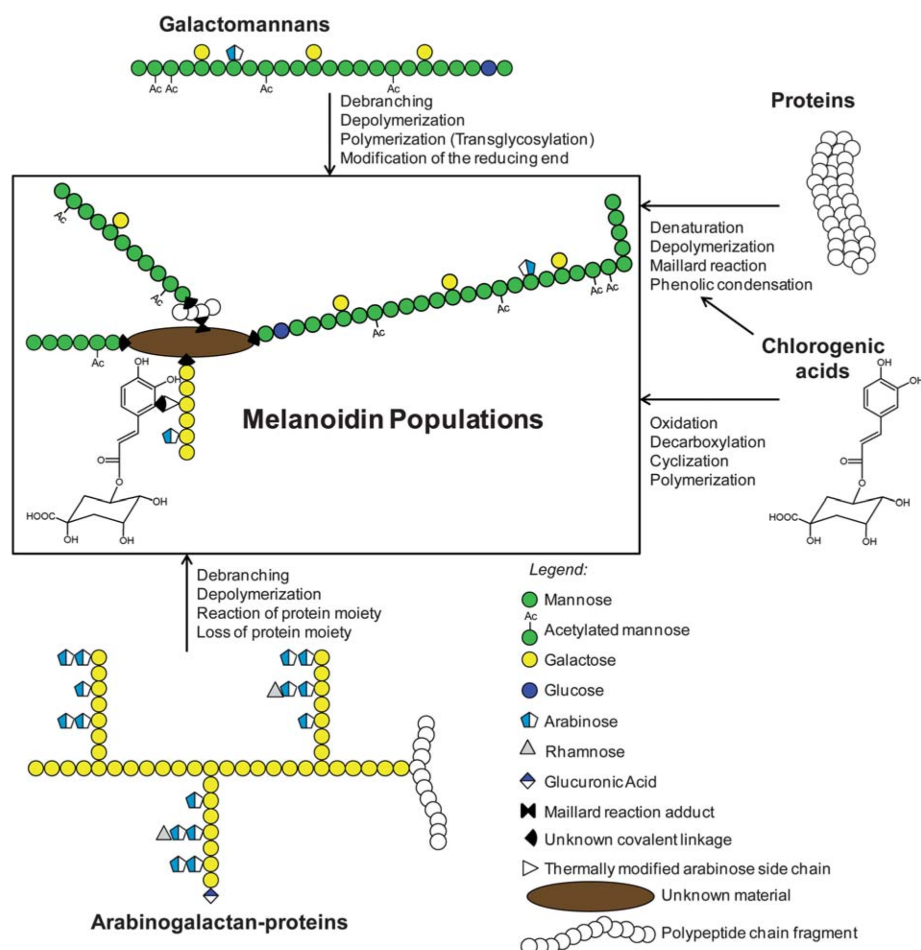

Fig. 5 Illustration of coffee melanoidin formation (an adaptation of the formation originally produced by Nunes and Coimbra).[6]

that of the remaining polymeric material, it has been suggested that, although melanoidins present antioxidant activity, an important contribution to the overall antioxidant activity of the HMWM is given by the low molecular weight compounds linked non-covalently to the melanoidin skeleton, such as chlorogenic acids.[29–31] The antioxidant activity of melanoidins was reinforced by the observation of *in vitro* antioxidant activity in melanoidin fractions not containing chlorogenic acids, isolated from the HMWM by gel permeation chromatography and characterized by 2D NMR analysis.[17] The mechanism of the antioxidant action of coffee melanoidins is still unclear. However, it has been assumed that it is based on their radical scavenging activity and/or their metal chelating capacity.[29,30]

The antioxidant activity of coffee melanoidins has been evaluated by measuring their radical scavenging activity against stable free radicals: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{•+}$), 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{•}$), and *N*,*N*-dymethyl-*p*-phenylenediamine radical cation ($DMPD^{•+}$). Among others, assays testing their ability to prevent lipid peroxidation have also been used.[17,21,29–31] Borrelli *et al.*[21] showed a decrease of the radical scavenging activity of melanoidins isolated by gel filtration chromatography from coffee brews towards $ABTS^{•+}$ and $DMPD^{•+}$ with the increasing of the degree of roast, but the ability to prevent linoleic acid peroxidation was higher in the dark-roasted sample. On the contrary, with respect to the results obtained by the $ABTS^{•+}$ assay, Delgado-Andrade *et al.*[30] showed that the antioxidant activity of melanoidins isolated by ultrafiltration from instant coffees was lower in the light-roasted sample. As for the melanoidin fractions isolated from coffee brews, different results have also been reported on the effect of roasting on the antioxidant activity of whole coffee brews. For example, Richelle *et al.*[90] reported that coffee antioxidant activity decreased with roasting, whereas in other studies maximum antioxidant activity was observed for the medium-roasted coffee.[91,92] The differences in the assay procedures may contribute to the different results reported by the different authors. However, the previously discussed differences in the composition of coffee melanoidin fractions, in which the different melanoidin populations may exhibit antioxidant capacities through different mechanisms, should also be taken into account to explain the different results. Better knowledge of melanoidin structures would benefit the understanding of their antioxidant properties.

In terms of possible implications on human health, the antioxidant activity of coffee melanoidins has been associated with protective effects against oxidative damages. The HMWM of a coffee brew isolated by ultrafiltration and digested by simulating gastrointestinal enzymatic digestion showed protective effects on cultured human hepatoma HepG2 cells submitted to oxidative stress, such as a decrease on the activities of antioxidant enzymes

Table 1 Synoptic table of the studies focused on biological activities of coffee brew melanoidins

Biological activity	HMWM isolation		HMWM fractionation	Assay(s) for biological activity	Findings	Ref.
Antioxidant activity	Gel filtration chromatography[a]	N/A[b,c]	—	ABTS, DMPD, DPPH, inhibition of linoleic acid peroxidation, and redox potential	ABTS$^{\bullet+}$/DMPD$^{\bullet+}$ scavenging ability decreased with the increasing of the roasting degree. The ability to prevent linoleic acid peroxidation was higher in the dark-roasted sample	21
	Ultrafiltration	10 kDa	Ultrafiltration (10 kDa) after incubation in 2 M NaCl	DPPH, ABTS, FRAP, and inhibition of (AAPH)-induced linoleic acid oxidation	>50% of the antioxidant activity is due to low molecular weight compounds linked non-covalently to the melanoidin skeleton.	29,30
	Ultrafiltration	10 kDa	Ultrafiltration (10 kDa) after gastrointestinal digestion and after incubation in 2 M NaCl	DPPH, ABTS, FRAP, ORAC, and HOSC	Low molecular weight compounds released after gastrointestinal digestion exerted the highest antioxidant activity.	31
	Dialysis	3.5 kDa	Gel filtration chromatography	Lipid peroxidation in a rat liver microsome biological system	HMWM exerted a higher antioxidant activity than that of low-MWM.	98
Inhibition of matrix metalloproteases	Ultrafiltration	10 kDa	—	Inhibition of MMP-1, MMP-2, and MMP-9 activities	IC_{50} values for medium roasting ranged between 0.2–1.1 mg mL^{-1} of HMWM.	36
Antimicrobial activity	Ultrafiltration	10 kDa	—	MIC values against bacterial strains of Gram-negative, Gram-positive, and Gram-negative that produce siderophores	MIC values ranged between 2–10 mg mL^{-1} of HMWM.	32,33
Modulation of the bacterial colon population	Stepwise ultrafiltration	100, 50, 10, and 3 kDa	Ultrafiltration (1 kDa) after incubation with human fecal bacteria	Microbial population analysis by FISH and DGGE	Growth of bacterial cells belonging to the *Bacteroides-Prevotella* group	94
Anticariogenic activity	Dialysis	3.5 kDa	Gel filtration chromatography	*Streptococcus mutans* adhesion to and detachment from saliva-coated hydroxypatite beads and biofilm formation	HMWM (6 mg mL^{-1}) exerted 91% of adherence inhibition, 23% of detached bacteria and 100% of biofilm formation inhibition	117
Anti-inflammatory activity	Dialysis	12–14 kDa	—	Analysis of inflammatory markers in liver samples from rats subjected to a high-fat diet	Reduction of proinflammatory cytokines and increase of anti-inflammatory cytokines in melanoidin-drinking rats	119
Antihypertensive activity	Ultrafiltration	10 kDa	Ultrafiltration (10 kDa) after overnight incubation in 2 M NaCl	ACE-inhibitory activity	HMWM (2 mg mL^{-1}) exerted 37–45% of ACE inhibition.	34
Antiglycative activity	Ultrafiltration	10 kDa	—	Inhibition of bovine serum albumin glycation with glucose	IC_{50} = 274 μg mL^{-1} of HMWM	35

[a] Method used for HMWM isolation. [b] Molecular weight cut-off (of the membrane or fiber used). [c] N/A, Not applicable. Other abbreviations used: ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); ACE, angiotensin-I converting enzyme; APPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; DGGE, denaturing gradient gel electrophoresis; DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FISH, fluorescence *in situ* hybridization; FRAP, ferric reducing activity power; (H)MWM, (high) molecular weight material; HOSC, hydroxyl radical scavenging capacity; IC_{50}, 50% inhibitory concentration; MIC, minimum inhibitory concentration; MMP, matrix metalloprotease; ORAC, oxygen radical absorbance capacity.

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(glutathione peroxidase and glutathione reductase), whose activities are increased when cells are submitted to oxidative stress.[93] Also, the high molecular weight fractions of coffee brews isolated by stepwise ultrafiltration and subjected to *in vitro* fermentation for 24h with human fecal bacteria showed antioxidant activity,[94] suggesting a possibly role of coffee melanoidins in the protection against radical stress in the colon, which is associated with the development of colon cancer.[95] The HMWM from instant coffee can also inhibit the formation of hydroperoxide free radicals, and secondary lipoxidation products during simulated gastric digestion of turkey meat.[96] The ability of melanoidins to inhibit the lipoxidation can contribute to their health benefits, since lipoxidation products are involved in the development of atherosclerosis and other diseases.[97] The inhibition of lipoxidation by coffee melanoidins was also reported to occur in a rat liver microsome system,[98] and model glucose–glycine melanoidins also exerted a protective effect against lipoxidation in isolated rat hepatocytes submitted to oxidative stress.[99]

4.2. Inhibition of matrix metalloproteases

Matrix metalloproteases (MMPs) are a family of *endo*-peptidases that are thought to play a central role in tumor growth and metastasis.[100] The potential activity of coffee melanoidins as MMP inhibitors was reported based on the ability of the HMWM isolated from coffee brews to inhibit the *in vitro* activity of selected human MMPs (MMP-1, MMP-2, and MMP-9). For all MMPs (and roasting times >10 min), IC_{50} values ranged between 0.2 and 1.1 mg mL^{-1}. The HMWM from green coffee brew showed no significant inhibitory activity against any of the MMPs at concentrations up to 2.5 mg mL^{-1}. Also, the inhibitory potential increased with the degree of roast.[36]

Concerning possible effects on human health, the inhibitory activity of coffee melanoidins may offer protective effects against colon cancer, since MMP-1, MMP-2, and MMP-9 are thought to be involved in the pathogenesis of colon cancer.[101] As estimated by Fogliano and Morales,[11] the daily intake of coffee melanoidins range between 0.5 to 2.0 g for moderate and heavy consumers, respectively. This allows to estimate, based on the assumption that the colon accumulates its content over at least 24 h in a maximum volume of 2 L,[36] a concentration of coffee melanoidins in the order of 0.25 to 1 mg mL^{-1} in colon. These values are comparable to the IC_{50} values obtained with the HMWM of (roasted) coffee brews, suggesting that the regular intake of melanoidins from coffee brews could have a MMP inhibitory activity and, consequently, be involved in the protection against colon cancer.

4.3. Antimicrobial activity

The antimicrobial activity of coffee melanoidin fractions (referring to the HMWM isolated by ultrafiltration from coffee brews) was evaluated against bacterial strains of Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella typhymurium*), including those that produce high-affinity iron chelating compounds (siderophores).[32,33] The antimicrobial activity was tested as the minimum inhibitory concentration (MIC), defined as the lowest concentration of melanoidin fractions that did not produce any detected cell growth.[32] For all strains studied, the MIC of coffee melanoidin fractions ranged between 2 and 10 mg mL^{-1}.[32,33] Gram-positive bacteria were more sensitive to the antimicrobial activity of coffee melanoidin fractions, showing lower MIC values (2–3 mg mL^{-1}) than Gram-negative bacteria ($\geq$4 mg mL^{-1}). As coffee brews, depending on the method of preparation, have an estimated melanoidin content ranging between 2 to 4 mg mL^{-1},[11] it can be inferred that all coffee brews present antibacterial activity in the mouth against these Gram-negative bacteria and possibly also against some Gram-positive. On the other hand, based on the estimated melanoidin concentration of 0.25 to 1 mg mL^{-1} in colon of moderate and heavy consumers (discussed in point 4.2), it can be expected that the regular intake of coffee melanoidins has an inhibitory/regulatory effect on colon microflora, but does not prevent microbial growth.

Comparing instant coffees with different degrees of roast, it was observed that the HMWM from the higher degree of roast exerts a higher inhibitory bacterial growing activity, measured against *Geobacillus stearothermophylus*. It was also observed, for all instant coffees, that the non-covalently linked compounds released from the HMWM after incubation in 2 M NaCl exert a lower antibacterial activity than that of the remaining HMWM.[102] In another study, it was showed that the antibacterial activity against *Escherichia coli* of low and intermediate molecular weight compounds isolated from a coffee brew by sequential ultrafiltration steps exert a lower antibacterial activity than that of the HMWM isolated in the first ultrafiltration (referred as melanoidins).[32] The antibacterial activity reported for the coffee melanoidins is in line with other studies demonstrating the antibacterial activity of whole coffee brews, though other compounds, such as caffeine and α-dicarbonyl compounds formed during the roasting process, have been suggested to contribute greatly for their antimicrobial activity against certain bacterial strains, such as *Salmonella enterica* and *Staphylococcus aureus*, respectively.[103–106] Maillard reaction products, obtained using sugar-amino acid model systems in solution, were also pointed out to possess antibacterial activity.[107–109] The high molecular weight Maillard reaction products were more inhibitory than the low molecular weight products when tested with *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*.[107]

The antimicrobial capacity of coffee melanoidins has been ascribed to their metal chelating properties.[32,33] In particular, three different mechanisms for the antibacterial activity of coffee melanoidins were suggested by Rufián-Henares and de la Cueva,[33] as follows: (1) at low concentrations, melanoidins may exert a bacteriostatic activity mediated by iron chelation from the culture medium; (2) in bacterial strains that are able to produce siderophores for iron acquisition, melanoidins may chelate the siderophore-Fe^{3+} complex, which could decrease the virulence of such pathogenic bacteria; and (3) coffee melanoidins may also exert a bactericide activity at high concentrations by removing Mg^{2+} cations from the outer membrane, promoting the disruption of the cell membrane and allowing the release of intracellular molecules. The mechanism behind the distinct sensitiveness of Gram-negative and Gram-positive bacteria is not yet known beyond the assumption that the absence in Gram-positive microorganisms of the outer membrane makes them more susceptible to antimicrobial substances.[33]

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No reported studies on the antimicrobial activity of melanoidins in yeasts or fungi are available.

4.4. Modulation of the bacterial colon population

In vitro fermentation of coffee brew high molecular weight fractions (>100, 50–100, 10–50, and 3–10 kDa) with human fecal bacteria showed that melanoidins are degraded or modified by the human gut bacteria, as indicated by the decrease of the absorbance at 405 nm of the solutions. For all coffee fractions, it was observed an increase of *Bacteroides-Prevotella*,[94] as had been observed in a previous study with an ethanol soluble high molecular weight fraction characterized by 2D NMR.[28] Because in these studies the melanoidins were probably present in mixtures with polysaccharides, it cannot be disclosed if this effect can be attributed only to the melanoidins. However, model melanoidins, prepared from a mixture of glucose and amino acid or protein, were shown to affect the growth of human gut bacteria, their cell numbers were increased or decreased depending on the bacteria and the time of thermal treatment or incubation.[110,111]

The capacity of coffee melanoidin fractions to modulate the bacterial colon population is in accordance with a human volunteer study demonstrating that the moderate consumption of an instant coffee produces an increase in the number of *Bacteroides-Prevotella* bacteria detected in faeces.[112] However, in this *in vivo* study, the main bacteria increase was observed for *Bifidobacterium* spp., known for their probiotic effects.[113,114] The selective modulation of the bacterial colon population by coffee consumers observed by this *in vivo* study is in accordance with the estimated amount of daily coffee intake discussed in point 4.3, providing melanoidins in the colon in concentrations not far but lower than their MIC, promoting the growth of the least sensitive.

4.5. Anticariogenic activity

Streptococcus mutans is a bacterial species known to be involved in the development of human dental caries. Its cariogenic potential is in part related to its ability to adhere to the tooth surface and form a biofilm.[115,116] The potential anticariogenic activity of coffee melanoidins was reported based on the ability of HMWM isolated from a coffee brew, and brown-colored melanoidin fractions derived from the HMWM, to affect *S. mutans* sucrose-dependent adhesion to and detachment from saliva-coated hydroxyapatite beads and to inhibit biofilm formation on microliter plates. The presence of HMWM at a concentration of 6 mg mL^{-1} inhibited the *S. mutans* adhesion to the beads in 91% and abolished the biofilm production. The bacterial detachment from the beads after 2 h incubation was three times higher with HMWM (23%) than with the controls (7%). Based on these results and those obtained from the melanoidin fractions, it was suggested that the coffee melanoidins may exert an anticariogenic activity.[117] This hypothesis was also supported by a previous study demonstrating that the anti-adhesive effect of coffee brews on the adhesive properties of *S. mutans* are due to both naturally occurring and roasting-induced molecules, the latter including melanoidins.[118] Also, based on the estimated melanoidin concentration in the coffee brews (2–4 mg mL^{-1}),[11] it is expected that coffee preparations promote dental caries protection.

4.6. Anti-inflammatory activity

Several inflammatory markers were quantified in liver samples from rats subjected to a high-fat diet for 3 months and to the ingestion of different beverages from the beginning of the second month. Rats drinking decaffeinated coffee or melanoidins (the HMWM isolated from the decaffeinated coffee), compared with control rats drinking water, showed reduced concentrations of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interferon-γ (IFN-γ) and increase of anti-inflammatory ones such as interleukin-4 (IL-4). These and other results obtained in this study suggested that coffee melanoidins may exert an anti-inflammatory activity, particularly in liver. Concerning possible implications on human health, it was suggested that the anti-inflammatory activity of coffee melanoidins may play a role in counteracting the progression of liver diseases, namely nonalcoholic steatohepatitis, a chronic inflammation state in which radical oxygen species and several immunomodulatory factor contribute to liver injury.[119] In agreement with this study, other studies conducted in mice suggested the anti-inflammatory action of coffee brews.[120,121]

4.7. Antihypertensive activity

The potential antihypertensive activity of the HMWM isolated by ultrafiltration from instant coffees was evaluated *in vitro* by monitoring the angiotensin-I converting enzyme (ACE)-inhibitory activity.[34] ACE (EC 3.4.15.1) is a circulating enzyme that catalyzes the cleavage of a dipeptide from the C-terminal of the decapeptide angiotensin I to form the potent vasopressor angiotensin II. It also inactivates the vasodilator bradykinin by sequential removal of two C-terminal dipeptides. ACE is a key element of the renin-angiotensin system that regulates blood pressure, and ACE inhibitors are important for the treatment of hypertension.[122] The HMWM isolated from instant coffees showed ACE-inhibitory activity (37–45% of ACE inhibition at a concentration of 2 mg mL^{-1}), being this activity attributed to the melanoidins. The high molecular weight fraction recovered after overnight incubation of the HMWM in 2 M NaCl showed much higher ACE-inhibitory activity (53–59%) than the low molecular weight fraction (12–20%). Also, it was observed that the ACE-inhibitory activity of HMWM fractions (containing the melanoidins) increase with the degree of roast.[34] In line with this study, another study showed that concentrations higher than 1.5 mg mL^{-1} of the HMWM isolated from coffee brews are necessary to inhibit *in vitro* ACE activity by 50%.[36] The antihypertensive activity of melanoidins was also suggested based on the study of the antihypertensive activity of Maillard reaction products obtained from several glucose-amino acid model systems in solution.[108] Based on the estimated intake of coffee melanoidins of 0.5 to 2.0 g per day,[11] their absorption and residence and dilution in the blood, it can be estimated that the amount of melanoidins from coffee intake are far from reaching the required concentration to have an effect on ACE inhibition.

The mechanism of action for potential ACE-inhibitory activity of melanoidins is not known, but different mechanisms have

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been suggested, as previously described by Rufián-Henares and Morales.[34,108] Because ACE is a zinc-dependent enzyme,[122] the inhibitory activity of melanoidins can come from their metal chelating properties. On other hand, melanoidins can act as an ACE non-competitive inhibitor, bind to the enzyme in an area other than the active center, deform the enzyme, and hinder binding to the substrate.[123]

4.8. Antiglycative activity

Advanced glycation end products (AGEs) are considered important mediators of diabetes complications. Thus, inhibitors of glycation reactions are of great interest because of their preventive or therapeutic potential.[124] The potential antiglycative activity of coffee melanoidins was reported based on the ability of the HMWM isolated from a coffee brew to inhibit *in vitro* glycation of bovine serum albumin with glucose. The concentration of the HMWM which is able to inhibit 50% of the glycation (IC_{50}) was 274 μg mL^{-1}. However, the accumulation of Amadori products during glycation in the presence of the HMWM was observed, suggesting that its antiglycative action is paramount in the post-Amadori phase of the reaction. The HMWM showed a lower antiglycative activity compared to the low molecular weight fraction (IC_{50} of 60 μg mL^{-1}).[35] Based on the estimated intake of coffee melanoidins of 0.5 to 2.0 g per day,[11] although dependent on the proportion of melanoidins absorbed and present in the blood, it can be estimated that the concentration of melanoidins in blood are in the range of the effective dose for a possible antiglycative activity of coffee brew.

4.9. Other biological activities

The reaction of methanol-insoluble fractions isolated from instant coffee (ascribed to melanoidin fractions) with nitrous acid and thiocyanate was studied under acid conditions simulating the mixture of coffee melanoidins, saliva, and gastric juice. Based on the results obtained, it was suggested that coffee melanoidins may react with salivary nitrite and thiocyanate in the gastric lumen, producing nitric oxide (NO). It was also suggested that the mechanism of reaction involves *o*-diphenol groups in melanoidins.[125] Concerning possible physiological effects, the formation of NO in the stomach may contribute to the inhibition of the microbial growth as well as the regulation of mucosal flow, mucosal formation and gastric mobility.[125]

Model melanoidins (>12 kDa) prepared from a glucose–glycine mixture (dry-heated for 2 h at 125 °C) exhibited modest but significant genotoxic effects in human lymphocytes cultures.[126] However, to the best of our knowledge, there is no study on coffee melanoidins reporting their genotoxic effects in human lymphocytes. This is an aspect that deserves future attention.

5. Conclusions

Future studies are needed to better understand the structures of coffee melanoidins, mechanisms of their formation, and their potential health impacts, since there are still several unanswered questions. With respect to their potential health impacts, most of the studies undertaken to date on biological activities of coffee melanoidins were developed using the high molecular weight material isolated from coffee brews without subsequent purification. However, it is known that different melanoidin populations (considering their structural features) are present in coffee brew. Thus, further studies are required to understand the relationship between the chemical structure of the different melanoidin populations and their biological activities. Also, it is important to confirm whether the biological activities attributed to coffee melanoidins based on *in vitro* studies are also observed in *in vivo* studies.

Beyond the antioxidant activity provided by coffee brews due to the melanoidins and other components, the studies published to date suggest that the amount of melanoidins ingested during regular coffee intake should provide protection against colon cancer by inhibition of matrix metalloproteases, which prevent bacterial growth in the mouth and the appearance of dental caries, promote selective bacterial growth in the colon, and exert anti-inflammatory and antiglycative effects.

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