Amino Acids (2012) 42:1097–1109
DOI 10.1007/s00726-010-0774-1

REVIEW ARTICLE

Physiological relevance of dietary melanoidins

Francisco J. Morales · Veronika Somoza · Vincenzo Fogliano

Received: 31 May 2010 / Accepted: 30 August 2010 / Published online: 15 October 2010

Abstract Melanoidins are the final products of the Maillard reaction. The main dietary sources of melanoidins are coffee, bread crust, bakery products, black beer and cocoa. Although the chemical structures of melanoidins are widely unknown, data from gravimetric techniques allow to roughly estimate a daily intake in the order of 10 g with a Western diet. Melanoidins contribute to the sensorial properties, modulating texture and flavour of foods. Growing evidence also suggests that melanoidins have health beneficial properties, such as chemopreventive, antioxidant and antimicrobial activities, and the ability to chelate different minerals. In the gastrointestinal tract, melanoidins behave not only as antioxidants, but also as dietary fibre by promoting the growth of bifidobacteria. This array of biological activities suggests the need for analytical techniques to identify the melanoidin structures and to control their formation during thermal food processing.

Keywords Maillard reaction · Dietary fibre · Coffee · Antimicrobial · Phase I and II enzymes

F. J. Morales
Instituto de Ciencia y Tecnología de los Alimentos y Nutrición, Consejo Superior de Investigaciones Científicas, José Antonio Novais 10, 28040 Madrid, Spain

V. Somoza
Research Platform Molecular Food Science, University of Vienna, Althanstrasse 14 (UZA II), 1090 Vienna, Austria

V. Fogliano (✉)
Dipartimento di Scienza degli Alimenti, University of Napoli "Federico II", via Università 100, 80055 Portici, Italy
e-mail: fogliano@unina.it

Chemical properties of melanoidins

Melanoidins are formed at the final stages of the Maillard reaction (MR). The different steps of the MR were illustrated for the first time in the general scheme by John Hodge (1953) who schematized the reaction in three steps: I, formation of early stage compounds, mainly the Amadori products; II; formation of intermediate Maillard reaction products (MRPs), such as hydroxymethylfurfural (HMF), Strecker aldehydes or pyrazines; III, formation of final reaction products, the melanoidins.

Melanoidins are brown in colour, have a heterogeneous structure, and different from caramel, they always contain nitrogen. They are considered high molecular weight polymers, although the term polymer might not be fully appropriate because of their heterogeneous nature. The estimation of the molecular weight of melanoidin molecular weight has been performed by gel filtration or dialysis (Hofmann et al. 2001), mass spectrometry (Smaniotto et al. 2009) and centrifugation through membranes with different cut-off sizes (Borrelli et al. 2003). However, dialysis membranes are calibrated using globular proteins, making any size estimations very rough. There is no general consensus about their actual molecular weight, ranging from a few thousands of Daltons (pre-melanoidins, Finot and Furniss 1989) to 10–20 kDa (melanoidins having intermediate molecular weight, Borrelli et al. 2003), to more than 100 kDa (Borrelli et al. 2002; Hofmann 1998).

Melanoidins are quite hydrophilic (Migo et al. 1997) and they are usually negatively charged (Morales 2002; Bekedam et al. 2007). In complex systems, other compounds are likely non-covalently associated to their surface (Delgado-Andrade and Morales 2005; D'Agostina et al. 2004) and this is another factor contributing to the analytical challenge to determine their molecular size.

Table 1 Chemical properties of melanoidins investigated in foods

Food melanoidins	Water solubility	Other components	References
Coffee	High	Chlorogenic acid	Bekedam et al. (2008d)
Soy sauce	Moderate	–	Wang et al. (2007), Homma et al. (1998)
Bread	Low	–	Borrelli et al. (2003)
Nut and seeds	Low	Fats	Açar et al. (2009)
Cocoa	Moderate	Catechins	Summa et al. (2008)
Milk	Moderate	–	Calligaris et al. (2004)
Dark beer	High	Phenolic compounds	Rivero et al. (2005)
Balsamic vinegar	Moderate	–	Falcone and Giudici (2008)
Sweet wine	Moderate	Phenolic compounds	Ortega-Heras and Gonzalez-Sanjosé (2009)

The solubility of melanoidins depends on the nature of the reactants and on the size of the polymer: Those having a very high molecular weight are often insoluble. Some categories of melanoidins according the nature of the reactants can be differentiated (Table 1). In protein-rich foods, the MR leads to the formation of a network among the proteins which are crosslinked by carbohydrate-derived structures. In this case, a particular class of melanoidins is formed: melanoproteins, having a very high molecular weight and being largely insoluble (Hofmann 1998).

A different situation occurs when low molecular weight (LMW) compounds such as monosaccharides and amino acids are involved in the polymer formation. In this case, the formation of melanoidins occurs by a bottom up mechanism with the formation of LMW blocks of brown soluble material. If the reaction is prolonged, these blocks will condensate, generating high molecular weight soluble melanoidins. However, under low water activity conditions, also a significant amount of insoluble material is formed (COST Action 919 1998).

When the reaction between amines and sugars involves polysaccharides and oligosaccharides, a variety of products can be formed, depending on the nature of the reactants. In starch-rich foods, the melanoidins are poorly soluble due to the limited solubility of the starch.

Next to proteins/amino acids and carbohydrates, other structures such as phenolic compounds can be incorporated into the melanoidins. This has been demonstrated for coffee melanoidins, where fragments of chlorogenic acid (CGA) have been analysed (Borrelli et al. 2002) and cocoa melanoidins containing polyphenols like catechins (Summa et al. 2008). The brown polymers in nuts and seeds seem to be of particular interest (Açar et al. 2009) since, during roasting and frying of these products, the fat moiety also plays a role in the formation of melanoidins. During heat treatment, fatty acids can be oxidized and the fat carbonyl group may react with amino group much more efficiently than the carbohydrate carbonyl group (Oliviero et al. 2009; Hidalgo and Zamora 2008). In these cases, it is likely that lipid oxidation products end up in a structure that contributes to the formation of an insoluble brown polymer.

As mentioned above, it is not possible to define a precise melanoidin structure due to their heterogeneous nature. However, some structural elements have been identified in model systems and in foods. Tressl et al. (1998) showed that the reaction between deoxy-D-ribose and methyl 4-aminobutyrate leads to the formation of linear oligomers that contain units of furan and pyrroles. These oligomers can form, through polycondensation reactions, high molecular weight melanoidins with repeating units. Similarly, Kato and Hayase (2002) suggested that melanoidins have structural similarities with Blue M1, a compound containing a pyrrolopyrrole ring which is formed in xylose–glycine reaction system.

Cammerer and Kroh (1995) proposed a melanoidin structure having a skeleton that is formed by polymerized carbohydrate degradation products and linked with amino compounds. They showed how the melanoidins structure can easily change with the reaction conditions. Later on, the same authors demonstrated that, if the reaction is carried out with oligosaccharides, intact carbohydrate structures are incorporated into the polymer, suggesting that the glycosidic bond remained intact, particularly in water-free conditions (Cämmerer et al. 2002). Using a radiolabelled approach, Brands et al. (2002) were able to determine an extinction coefficient of melanoidin and showed that, in a glucose casein system, the amount of sugar bound to casein is directly proportional to the colour of the solution.

In foods, melanoidins are more frequently built up with proteins than free amino acids. Hofmann (1998) showed that, in casein glucose systems, LMW coloured substances can be formed on the side chain amino groups of lysine or arginine, thus crosslinking different polypeptides. Alternatively, in systems like gluten, where proteins form a network through disulphide bridges, coloured small molecular weight MRPs (which are formed through ammonia release by glutamine and asparagines) can remain

physically entrapped in the gluten matrix (Fogliano et al. 1999).

Among food melanoidins, those from coffee are by far the most widely investigated. During roasting of green coffee beans, chemical and structural changes occur where polysaccharides, proteins and phenolic compounds contribute to the formation of coffee melanoidins (Viani and Illy 1995). Nunes and Coimbra (2007) proposed three different fractions in the high molecular weight fraction of coffee brew: melanoidins, galactomannan-like polysaccharides and arabinogalactan-like polysaccharides. Recently, it was also demonstrated that phenolic compounds can also be non-covalently linked to coffee melanoidins and melanoidins could act as carriers of LMW substances (Delgado-Andrade and Morales 2005).

With a series of six papers, Bekedam et al. (2006, 2007, 2008a, b, c, d) demonstrated that coffee melanoidins incorporate polysaccharides, CGAs, sucrose and amino acids/protein fragments. Prolonged roasting led to formation of melanoidins with high molecular weights, in which arabinogalactans are more involved than galactomannans. The authors hypothesized that chromophores may be formed or attached through the arabinose moiety of arabinogalactan proteins.

From NMR spectroscopy data, Bunzel and co-workers (Gniechwitz et al. 2008a, b) concluded that intact caffeic and ferulic acid derivatives are not incorporated into the melanoidins to a significant extent, suggesting that also phenolic structures were significantly modified during the reaction with other components during roasting.

In summary, the absence of a known molecular structure and the strict dependence of its concentration on processing conditions in the final product has hampered an estimation of the dietary intake of melanoidins thus far. However, mounting evidence suggests that melanoidins are not an inert material and that they may exert some physiological activity.

Melanoidins occurrence in foods

Since genesis of melanoidins is linked to the thermal processing of foods, it is expected that their occurrence is ubiquitous in our diet. However, there is a gap in the scientific literature concerning the estimation of the contents of melanoidins in different food stuffs. Even a rough estimate is missing due to the absence of reference materials. Noteworthy, not a single food melanoidin structure has been isolated and fully characterized so far, only some information regarding the major compounds contributing to the backbone structure of melanoidins is available.

However, the first melanoidins studied were those in soy sauce (Sakurai et al. 1981) due to their outstanding importance at the Asiatic diet. Several processed foods have been studied for their melanoidin content so far, either based on the abundance of melanoidins or based on the frequency of consumption: roasted coffee beans (Maier and Buttle 1973), cocoa (Hofmann et al. 1999), malt (Obretenov et al. 1991), roasted barley (Milic et al. 1975), black beer (Kuntcheva and Obretenov 1996), roasted potatoes (Ponnampalam and Mondy 2006), roasted pulses and seeds (Açar et al. 2009), roasted meat (Obretenov et al. 1993), dairy products (Meshcheryakova et al. 1987), bread (Borrelli et al. 2003), balsamic vinegar (Giudici et al. 2009), sweet wine (Ortega-Heras and Gonzalez-Sanjosé 2009), processed tomatoes (Adams et al. 2005). As mentioned before, coffee brews and instant coffee have been most widely studied due to the large amounts of water-soluble melanoidins formed.

As mentioned before, the main drawback to calculate food content of melanoidins is the lack of a reference material, the absence of a known molecular structure, and the appropriate analytical method. In the past, the content of melanoidins has been calculated gravimetrically, as the difference of the sum of known constituents of the target food (Obretenov and Vernin 1998). However, this procedure bears much bias. Later on, and based on the their polymeric nature, melanoidins were isolated from the LMW material of the food matrix and further characterized by their brown colour as measured for their absorbance at wavelength between 400 and 450 nm (Borrelli et al. 2002; Brands et al. 2002). A step forward was settled by the European COST Action 919 (COST 1999) within which analytical procedures of isolation and characterization were compared and harmonized, including functional properties attributed to melanoidins.

Briefly, there are three main approaches for isolation of melanoidins: extensive tubing dialysis, ultrafiltration, and gel permeation/molecular exclusion chromatography techniques. Figure 1 depicts a classical chromatographic separation of water-soluble coffee brew melanoidins. After this separation, melanoidin fractions are lyophilized and their content can be expressed in a dry mass basis of the initial food. For example, the melanoidin content in roasted coffee is expected to be up to 35% of their weight but it depends on the extent of the roasting and type of process (Bekedam et al. 2008b; Nunes and Coimbra 2007). An educated extrapolation considering the dietary intake from a moderate coffee consumption habits suggests an intake of melanoidins close to 0.5 g/day (Morales and Fogliano, unpublished results). A similar approximation can be calculated for bread and cereal products by combining the mean level of consumption with a weighted estimation of the melanoidin content of the product. In case of cereal products, melanoidins are mainly present in the crust, whereas in dry biscuits, they are distributed through the entire product. Due to the proteinaceous nature of those melanoidins, enzymatic treatment with proteases is

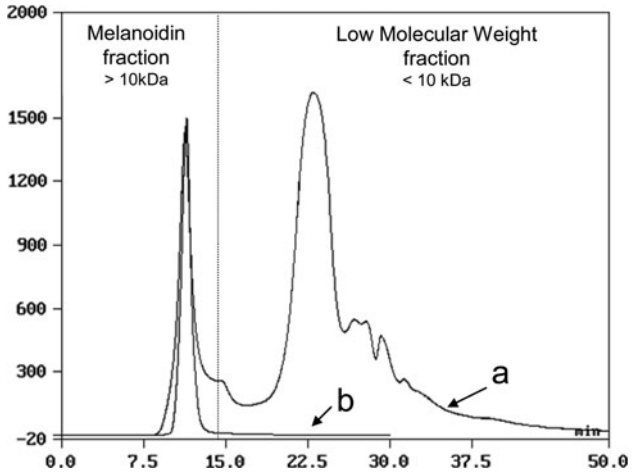

Fig. 1 Elution profiles of coffee brew (*a*) and its corresponding isolated melanoidin fraction (*b*) by size exclusion chromatography. Melanoidin fraction is isolated by ultrafiltration. *Dotted line* denotes the nominal cut-off at 10 kDa

necessary before isolation and quantitation (Borrelli et al. 2003). For bread and biscuits, an intake up to 15 and up to 8.5 g/day can be estimated, respectively. Those numbers for the intake of melanoidins from coffee and bread suggest a total dietary intake of about 10 g/day for the general population consuming a classical western diet (Morales and Fogliano, unpublished results).

In vivo effects of melanoidins

A prerequisite for dietary compounds to have a biological effect is its bioavailability, with the relevant exception of the effect inside the gastrointestinal tract. In general, the bioavailability of high molecular weight MRPs is assumed to be highly limited, whereas for low molecular MRPs, in particular when administered as free, non-protein linked compound, substantial amounts can be detected in the urine after dietary intake (Somoza 2005).

The first metabolic transit data of fructoselysine as an Amadori compound in human volunteers have been reported by Erbersdobler et al. (1986), showing that urinary excretion of orally administered fructoselysine is about 3%, whereas faecal excretion can be estimated at about 1%. These data confirmed a hypothesis made 16 years before (Erbersdobler et al. 1970), when intestinal microorganisms were thought to degrade Amadori products. The proof of this hypothesis was finally provided by Wiame et al. (2002) who discovered the enzyme fructosamine-6-kinase, which enables *Escherichia coli* and other species to metabolize Amadori products.

In the past 20 years, a few more studies focused on the metabolism of food-derived MRPs (for review, see Faist and Erbersdobler 2001; Bergmann et al. 2001; Somoza et al. 2005, 2006), all demonstrating an absorption rate of LMW MRPs of up to 30%, depending on the structure and the administered dose. Bergmann et al. (2001) used positron emission tomography (PET) to monitor biodistribution and elimination of radiofluorinated *N*ε-carboxymethyllysine (CML). The authors found that the compound was rapidly absorbed and transported into circulation, followed by an excretion through the kidneys.

For high molecular weight melanoidins, Finot and Magnenat (1981) reported an urinary excretion rate of a 10,000 Da fraction of a ^{14}C-labelled, at 100°C heated, casein/glucose mixture of 4.3%, as opposed to a 27% urinary excretion of the administered dose for the LMW fraction below a molecular weight of 10,000 Da. Taken together, there is circumstantial evidence that low and even high molecular MRPs are being absorbed at least to some extent, albeit metabolic transit data for a MRP structure showing a structure-specific bioactivity such as an antioxidant activity in vivo are still lacking. For heat-treated foods, however, numerous intervention trials in animals and humans have been performed aiming at their ability to increase the antioxidant capacity in plasma or tissues.

Regarding melanoidins, studies on rats suggested that they are absorbed, probably after some modification by intestinal enzymes and/or microorganisms. Interestingly, in studies performed by Finot and Magnenat (1981), a LMW fraction was hardly detected by gel chromatography in the faeces of rats fed a diet containing 2% HMW melanoidins, but the melanoidins excreted had an even smaller proportion of LMW components than the original one. Therefore, it may be speculated that even the HMW melanoidin fraction in the diet was degraded into LMW fractions by intestinal microorganisms, and these products were absorbed in the intestines. However, even non-absorbed melanoidins may elicit beneficial effects passing through the intestines (Gokmen et al. 2009).

Melanoidins acting as dietary fibre

The daily intake of melanoidins from different sources is in the order of magnitude of the amount recommended for dietary fibre intake which is of 30 g/day. Unfortunately, melanoidins do not fall into the definition of dietary fibre because they are not exactly "polysaccharides naturally present in raw foods" as they are formed upon processing and moreover they include an amino acidic/protein moiety besides polysaccharides.

On the other hand, there is mounting evidence that melanoidins actually behave as dietary fibre, being largely indigestible by humans and fermented in the gut. Comparing melanoidins with the various types of natural polysaccharides, a structural similarity with cereal

arabinoxylans, can be highlighted. In fact, this particular dietary fibre is predominantly linked to phenolic compounds, mainly to ferulic acid, and so, like melanoidins, arabinoxylans have an antioxidant capacity close to 100 mmol Trolox equivalents/kg (Serpen et al. 2008).

Cereal dietary fibre was considered as antioxidant dietary fibre (Saura-Calixto 1998) and the relevant physiological role that this type of food material can exert in vivo has been outlined in different papers (Vitaglione et al. 2008; Gokmen et al. 2009). From a functional standpoint, melanoidins perfectly fit into the definition of the antioxidant dietary fibre. Due to the abundance of reducing functional groups present in this polymer, both soluble and insoluble melanoidins can act like antioxidant dietary fibre, quenching radical species which are continuously formed in the gastrointestinal tract (Babbs 1990). The quantitative relevance of melanoidins in maintaining the reducing environment in the gut should be a matter of future assessment. However, the observation by Garsetti et al. (2000) reporting that the antioxidant capacity of faeces is related to the coffee consumption and not to the intake of whole grain, fruit and vegetables suggests that melanoidins, at least those from coffee, may have a prominent role in this respect.

The other fundamental feature that should be investigated for any non-digestible food ingredient is the ability to promote the growth of beneficial bacteria in the lower gut. This important property is attributed to prebiotic compounds in foods. In general, the term 'prebiotic substance' refers to soluble dietary fibre (fructo-oligosaccharides and inulin) that is able to modify the balance of intestinal microorganisms, mainly increasing the concentration of lactobacillus and bifidobacteria (Gibson et al. 2004).

Data from human and animal studies have shown that dietary melanoidins are not digested in the upper gastrointestinal tract and they are mainly recovered in the faeces (Faist and Erbersdobler 2001). Therefore, food melanoidins, as part of the food's indigestible material that reaches the lower gut, might be metabolized by the gut microorganisms and might be considered as a potential prebiotic material. In this respect, first investigations were addressed by Erbersdobler et al. (1970) and Horikoshi et al. (1981) on the effect of MRPs on the microorganisms in rats. Later studies showed that melanoidins escape digestion and pass through the upper gastrointestinal tract where they can interact with the different microbial species present in the hindgut (Finot and Magnenat 1981).

Ames et al. (1999) tested the prebiotic activity of glycated BSA, but no beneficial effects were found. Later on, Dell'Aquila et al. (2003) showed that low molecular mass products formed by peptic and tryptic digests of melanoproteins are able to change the growth of bacteria.

Borrelli and Fogliano (2005) investigated the potential prebiotic activity by a static batch culture of different melanoidins isolated from bread. Results showed that anaerobic bacteria, particularly bifidobacteria strains, are able to use bread crust melanoidins as unique carbon source. The bacterial growth was different for the various types of melanoidin samples, indicating that the starting material and the processing conditions have a strong influence on the prebiotic potential of bread melanoidins. In particular, it was possible to note that after about 10 h of fermentation, the resulting bread melanoidins well support bifidobacteria growth, while the other bacteria considered (Clostridia, *Bacteroides* spp., *Streptococci*, and Enterobacteriacea) showed a limited aptitude to use those melanoidins. In conclusion, bread crust melanoidins can be metabolized/fermented by the human hindgut microorganisms. These melanoidins selectively enhance the growth of bifidobacteria, which are desirable bacteria in the gut due to their health-promoting properties.

A similar effect could be observed for coffee silverskin, which is a by-product of coffee roasting that contains very high contents of coffee melanoidins (Borrelli et al. 2004). Nunes and Coimbra (2007) also demonstrated that melanoidins from coffee which are mainly constituted by polysaccharides such as arabinogalactans and galactomannans have a good prebiotic potential.

Recently, a series of papers published by the group of Bunzel from the University of Minnesota (Gniechwitz et al. 2008a, b; Reichardt et al. 2009) showed that coffee melanoidins definitively behave as a soluble dietary fibre since they are fermented by the microorganisms in the gut. High amounts of acetate and propionate were produced after microbial degradation of high molecular weight components from coffee, the authors also point out that most of the effect is due to galactomannans and arabinogalactans which are also present in the high molecular weight coffee fraction.

Altogether, data available up to now suggest that polysaccharide-rich melanoidins such as those present in coffee are preferentially metabolized by bifidobacteria, while protein-rich melanoidins as obtained by protein glucose mixtures are good substrates for potentially harmful protein metabolizing bacteria predominantly present in the descending tract of the colon.

The biological activities may also be attributed to the degradation products of melanoidins in the large intestine: As for other insoluble, non-digestible material, they may also play a role in the binding and/or release of other, putatively harmful dietary components.

Melanoidins as antimicrobial agents in foods

Thermal processing is basically applied to foods to increase their shelf-life, to promote a number of physical–chemical modifications that make the food edible and to increase the

acceptability of foods to consumers. Hence, the ability of melanoidins to influence food shelf life also by inhibiting undesired microbial growth is of great interest.

The antimicrobial activity of melanoidins was initially studied using model systems constituted by sugars and amino acids, and coffee. The most extensive study was performed by Einarsson et al. (1983) who measured the antimicrobial activity of arginine–xylose and histidine–glucose MR mixtures. Further investigations were carried out in specific microbial growth media, which showed that melanoidins can either stimulate microbial growth (Jemmali 1969) or inhibit it (Einarsson et al. 1983; Stecchini et al. 1991), but differences between low and high molecular weight products were not depicted. Daglia et al. (1994) reported that the antibacterial activity of coffee melanoidins depends on the degree of roasting and is not related to the procedure applied to prepare the coffee beverage. In summary, melanoidins can exert their antimicrobial activity mediated by a bacteriostatic activity or bactericidal activity. Basically, it depends on the melanoidin concentration, the pH and the temperature of the media, and the molecular weight and structure of the MRP. Antimicrobial activity of the MRP in study was mediated by lowering the iron solubility, resulting in a decrease in glucose and oxygen uptake. However, it can be assumed that the availability of other nutrients, which are essential for growth and survival of bacteria, is reduced. In addition, the antimicrobial activity of MRPs is also referred to the interference with the uptake of serine, glucose, and oxygen (Einarsson 1987), to inhibit the carbohydrate catabolizing enzymes of the microorganisms (Lanciotti et al. 1999) or their potential antioxidant activity (Mattila and Sandholm 1989). But the exact mechanism by which melanoidins or MRPs affect the bacterial growth is not completely known.

Recently, new insights were described by Rufián-Henares and Morales (2008a, b) for coffee and biscuit melanoidins of different molecular weight. The authors stated that melanoidins provoke irreversible cell membrane disruption, which was independent of the bacterial transmembrane potential. These results indicate that melanoidins are able to kill pathogenic bacterial strains (*E. coli*) by causing irreversible changes to the inner and outer membranes. This result indicates interference with biosynthetic processes, such as the inhibition of nutrient transport and macromolecular precursors. Rufián-Henares and De la Cueva (2009) suggested three different mechanisms for the antimicrobial activity of coffee melanoidins that can occur simultaneously. At low concentrations, melanoidins may exert a bacteriostatic activity mediated by iron chelation. Second, in bacterial strains that are able to produce siderophores for iron intake, melanoidins may chelate the siderophore–Fe^{3+} complex, which could decrease the virulence of pathogenic bacteria. Third, 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.

Opposite to the strategy to exert the antimicrobial activity of melanoidins towards specific strains such as *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus* or *Bacillus stearothermophilus*, Rufián-Henares and Morales (2006) developed a general procedure to estimate the antimicrobial activity of melanoidins. This approach allows to study the mechanism of action and to assess the antimicrobial potency of melanoidins independently of the specific strain. The target microorganism was *Geobacillus stearothermophilus* var. *calidolactis*, which is highly sensitive to a wide variety of antimicrobial substances. The efficiency of the antimicrobial activity exerted by the different melanoidins was expressed as oxytetracycline equivalents. In general, antimicrobial activity of melanoidins is significantly higher towards *S. aureus* (Gram-positive) than *E. coli* (Gram-negative), probably because Gram-positive bacteria are known to be more resistant to the action of antibiotics in a general way. Results indicate that both strains have different sensitivity against the presence of melanoidins and probably different mechanisms of inhibition. This procedure is presented as a rapid screening of the potential antimicrobial properties of melanoidins, and subsequently to MRPs as well, against pathogenic strains in order to isolated substances with biological activity.

Melanoidins acting as antimicrobial agents

Hiramoto et al. (2004) demonstrated the inhibitory activity of a variety of protein-derived melanoidins on urease-gastric mucin adhesion of *Helicobacter pylori* which plays an important role for the infection and colonization of the host (animals and humans). *H. pylori* infection is the primary cause of peptic ulcer and gastric cancer and its colonization in the stomach is suppressed by anionic polymers such as food melanoidins. Some beverages and foods protect tooth surfaces against *Streptococcus mutans* by inhibiting their glucosyltransferase activities. Adhesion of *S. mutans* to the tooth surface is an important step in the initiation and the development of dental caries. Results from Daglia et al. (2002) indicated that coffee might help to prevent cavities. Melanoidins may play a role in regulating dental plaque formation in vivo and, thereby, have long-term effects on the development of dental caries. It was also found that green, unroasted coffee beans were less active than the corresponding roasted coffee beans. But the effect indicating that coffee, if consumed solely, has anti-caries activity, but in the presence of additives the antibacterial and anti-caries activity was totally minimized.

Melanoidins as chelating agents

Melanoidins are likely to play an important role in the binding of nutritionally important metals (O'Brien and Morrissey 1997), potentially undesirable dietary compounds (Yen and Hsieh 1994) and odourants (Hofmann and Schieberle 2002). Secondly, the chelating properties towards metal ions also contribute to the antioxidant and antimicrobial properties of melanoidins in foods (Rufián-Henares and De la Cueva 2009) and in vivo (Tagliazucchi et al. 2010). Also, the chelating ability of melanoidins is relevant from technological (Petracco et al. 1999), nutritional (Jägerstad et al. 2002) and physiological (Faist and Erbersdobler 2001) points of view.

Chelation of odorants

This property has been used for the stabilization of aroma compounds, such as furfural or pyrrole derivatives and aromatic thiols, which have an impact on the overall organoleptic properties of coffee, beer and sweet wine (Kuntcheva and Obretenov 1996; Rivero-Pérez et al. 2002). Hofmann et al. (2001) studied the flavour binding properties of coffee melanoidins with different coffee aroma compounds in the same concentration as determined in a coffee brew. Melanoidins were remarkably active against thiols such as 2-furfurylthiol, 3-mercapto-3-methylbutyl formate, and 3-methyl-butene-1-thiol, but aldehydes remained unaffected. In addition, the presence of melanoidins is directly associated with the reduction of the intensity of the sulfury-roasty odour quality of the coffee brew. Melanoidins behave as multifunctional structures and are able to link covalently or ionically different odorant via Schiff bases, disulfide links, forming thioesters or thioacetals. Ortega-Heras and Gonzalez-Sanjosé (2009) established the impact of the addition of melanoidins from boiled must on the aroma and colour of sweet wines. The extent of the aroma binding process was reversible and variable depending on the kind of melanoidins present in the syrup. This aspect has a technological impact since flavour retention capacities of melanoidins could maintain or even improve the organoleptic properties of products such as roasted and instant coffee, bread, fruit juices or beer for a long time (Obretenov et al. 2002).

Chelation of ions

At pH values close to those found in most foods, melanoidins have a negative net charge and are able to bind metallic ions (Migo et al. 1997). Gomyo and Horikoshi (1976) reported that the melanoidins behave as anionic hydrophilic polymers, which can form stable complexes with metal cations. In general, melanoidins exert a net negative electric charge at pH 5.0, and melanoidins become more negative at higher pH values. Then, an equilibrium is reached when the rate of formation of the melanoidin–metal adduct is equal to the rate of dissociation. This property has been pointed out to exert negative nutritional consequences since, for instance, excessive coffee consumption will inhibit iron absorption (Mok et al. 1983) or calcium absorption.

Further melanoidin–metal-chelating activities of Ca(II), Fe(II), Zn(II), Pb(II), Cu(II) and Co(II) have been described (Wijewickreme et al. 1997; Rendleman 1987; Borrelli et al. 2002). In general, the metal-chelating activity is more effective of melanoidins obtained at more severe heating conditions that generate more insoluble structures (Rendleman 1987). The first evidence of an interaction between the mineral metabolism and MRPs was described in patients to whom sterilized solutions of amino acids and glucose were administered. In these patients, urinary excretion of Zn, Cu and Fe was abnormally high (Stegink et al. 1981) and clearly indicates negative health effects. On the other hand, the metal-chelating properties of coffee, barley coffee and dark beer has a positive physiological effect by inhibiting the lipid peroxidation during gastric digestion (Tagliazucchi et al. 2010). Negative nutritional consequences have to be evaluated in the context of a complex diet, where potential benefits and risks should be considered at the same time.

There is no consensus on the mechanism by which melanoidins exert their ion chelation abilities. First investigations pointed out that the active residues for the metal-chelating properties of melanoidins have been attributed to ketone or hydroxyl groups of pyranone or pyridone residues (Hashiba 1986). Also, the type of sugar was shown to be a significant parameter for obtaining melanoidins with high iron ability, and glucose was more efficient than lactose. However, chromophore residues might not be the main co-ordination sites for iron complexation in the melanoidin structure since browning and iron chelating ability are not related (Morales et al. 2005). Nunes and Coimbra (2007) investigated the chelating properties of different coffee melanoidins and suggested that the chelating ability is modulated by the carbohydrate composition. Wijewickreme et al. (1997) have shown that the Cu^{2+}-chelating ability of melanoidins is influenced by the type of sugar and the reaction conditions used for the synthesis. Borrelli et al. (2002) reported that synthetic melanoidins prepared from a glucose–glycine model mixture have better affinity towards Cu^{2+} than Pb^{2+} and Fe^{2+}. Later, metal-chelating properties of coffee melanoidins have been related to the presence of the degradation products of CGA in the polymer structure (Takenaka et al. 2005).

Chelation of LMW substances

According to their multifunctional properties, melanoidins are also able to bind other food components than metal ions. For instance, melanoidins are able to bind harmful compounds formed during food processing like heterocyclic amines (Solyakov et al. 2002). Heterocyclic amines are a group of structures which are formed during home cooking and industrial processing of protein-rich foods and exert a potent mutagenic activity. The International Agency for Research on Cancer has classified some heterocyclic amines as probable human carcinogens. Melanoidins were specifically active towards PhIP, harman, norharman, Trp-P-1, Trp-P-2, A-alpha-C and MeA-alpha-C. In other cases, melanoidins act as carriers of beneficial substances such as natural antioxidants. One example is the occurrence of non-covalently linked CGA likely by ionic interactions in coffee melanoidin fractions isolated by ultrafiltration (D'Agostina et al. 2004; Delgado-Andrade and Morales 2005). Although it has not been probed yet if CGA remains biologically available after gastrointestinal digestion, it is expected that CGA could be released from weak ionic link at the gastrointestinal environment.

Melanoidins acting as antioxidants in vivo

The prerequisite for dietary MRPs to exert antioxidant effects in vivo is that these compounds are absorbed in the gastrointestinal tract and are either transported in its parent structure to the target tissues or cell in effective concentrations or, prior to that, transformed into antioxidants.

The most recent evidence that food-derived MRPs elicit antioxidative properties in humans has been reported by Dittrich et al. (2009). The authors administered diets poor and rich in MRPs to eight healthy volunteers for 3 weeks in a weekly turn. The diet rich in MRPs contained dark beer, bread crust, and roasted coffee and led to a statistically significant increased oxidative stability by 35.5% of isolated LDL against copper-induced oxidation ex vivo. Although the experimental design of this study does not allow to draw conclusions about the active principles, the fact that administration of severely heat-treated foods versus mildly heat-treated foods resulted in an increased oxidative stability of LDL strongly supports the hypothesis that dietary MRPs exhibit antioxidant properties in vivo.

Coffee is one of the most commonly studied foods for the formation of MRPs and their biological effects, probably due to the intense heat treatment of the raw coffee beans during roasting and its widespread consumption. Given the fact that most of the naturally present phenolic antioxidants are degraded during roasting, the overall antioxidant activity of coffee is mainly attributed to high molecular weight MRPs, i.e. the melanoidins (Borrelli et al. 2003; Daglia et al. 2004). Anese and Nicoli (2003) studied the influence of some technological variables on the changes of the antioxidant capacity of ready-to-drink coffee brews and demonstrated a higher redox potential for brews prepared from dark roasted versus medium and light roasted coffee beans. When coffee beans are roasted with the addition of sugar (so-called *torrefacto* roasting), the antioxidant activity is even higher than that of conventionally roasted coffees, probably due to the increased formation of MRPs/melanoidins (López-Galilea et al. 2007).

When coffee brews were administered to human volunteers, the total antioxidant capacity of the plasma increased shortly after consumption. Natella et al. (2002) reported a statistically significant 7% increase in the plasma antioxidant capacity of blood samples drawn from healthy volunteers 2 h after intake of 200 ml regular coffee beverage. These results are in accordance with those reported by Esposito et al. (2003), demonstrating an increasing effect on the main water-soluble antioxidant in the plasma, glutathione, after administration of five cups of regular coffee per day to healthy volunteers for 1 week.

Bichler et al. (2007) reported a protection of human lymphocytes against DNA damage induced by reactive oxygen radicals or heterocyclic aromatic amines. Human volunteers consumed 600 ml of regular coffee per day for 5 days. Blood samples were taken before and after coffee consumption and cultivated in the presence of H_2O_2 or Trp-P-2, after which DNA damage was assessed by means of the comet assay (single cell gel electrophoresis). DNA damage was significantly reduced after coffee consumption, as compared to the induced damage before the intervention. The analysis of the cytosolic enzymes of the lymphocytes after coffee consumption showed an increased activity of superoxide dismutase (+38%), whereas glutathione peroxidase remained unchanged.

One of the active principles in coffee brews acting as key antioxidants might be *N*-methylpyridinium, a decarboxylation product that is generated during coffee bean roasting (Fig. 2; Stadler et al. 2002). In rats, Somoza et al. (2003) demonstrated an increase in total antioxidant capacity and alpha-tocopherol concentrations in the plasma of rats fed on coffee or *N*-methylpyridinium for 10 days. Further investigations on the cellular effects of *N*-methylpyridinium by Böttler et al. (2010) showed that it induces the protein expression and the gene transcription of Nrf2, a nuclear transcription factor that is responsible for the gene expression of antioxidant and detoxifying enzymes.

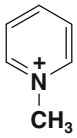

Fig. 2 Chemical structure of *N*-methylpyridinium (Somoza et al. 2003)

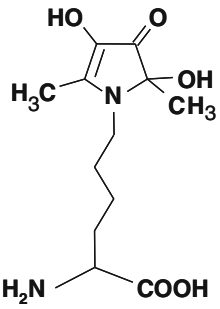

Fig. 3 Chemical structure of pronyl-lysine (Lindenmeier et al. 2002)

Next to *N*-methylpyridinium, there might be other coffee-derived antioxidants yet not identified, e.g. melanoidins (Goya et al. 2007). Although melanoidins comprise high molecular weight compounds, unlikely to be absorbed to exert antioxidant activities in vivo, these compounds might be degraded upon intestinal digestion, resulting in absorbable structures with antioxidant effects. Rufián-Henares and Morales (2007) reported that LMW compounds released by gastrointestinal digestion from a coffee melanoidin fraction with a molecular weight > 10 kDa exerted high antioxidant activities, even higher than those compounds bound ionically to melanoidins. Thus, gastrointestinal digestion is able to modify coffee melanoidins to some extent, either by modifying/releasing the ionically bound compounds and/or by genesis of new structures from the melanoidin skeleton after enzymatic treatment.

Beer is also rich in MRPs and melanoidins due to the malting and brewing process. The antioxidant properties of beer were reported to correlate both with the level of total polyphenols and with the content of melanoidins. Rivero et al. (2005) studied the antioxidant capacity of blond, dark and alcohol-free lager beers, showing that the oxidative damage of calf thymus DNA resulting in the formation of 8-OH-dG after induction by copper/ascorbic acid was prevented by co-incubation with the various beers, of which the darkest beer was most effective. When malt (5%, w/w) was fed to rats for 15 days, plasma contents of total tocopherols increased by 14%, while the levels of thiobarbituric acid reactive substances decreased (Somoza et al. 2005). In this study, the antioxidant effect of malt was demonstrated in vivo for the first time. Further investigations by Somoza and colleagues revealed that one of the key antioxidants in malt is pronyl-lysine (Fig. 3), which had earlier been identified as the most effective antioxidant formed in bread crust by means of cell culture experiments and an animal feeding trial (Lindenmeier et al. 2002).

Melanoidin as modulators of Phase I and Phase II enzymes

Detoxification enzymes generally function to minimize the potential damage from xenobiotics living organisms are exposed to, such as pharmaceuticals or food components. Many of these compounds show little relationship to previously encountered compounds or metabolites and most of them are of non-polar structures which do not allow urinary excretion. In order to prevent xenobiotics from being accumulated in tissues, a highly complex system of detoxifying enzymes has evolved to transform these compounds into polar, water-soluble structures that can be easily excreted through the urine.

In the literature, there is considerable evidence for an association between impaired detoxification and the risk for various diseases, such as some types of cancer, Parkinson's disease or chronic immune dysfunction syndrome. On the other hand, the intake of fruits and vegetables containing compounds that induce detoxification enzymes, such as antioxidants, has been shown to lower the risk for, e.g., colorectal cancer.

The biotransformation of xenobiotics occurs in two phases: functionalization, which uses oxygen to form a reactive site, and conjugation, which results in addition of a water-soluble group to the reactive site. These two steps, functionalization and conjugation, are termed Phase I and Phase II detoxification, respectively. Phase II conjugation reactions generally follow Phase I activation, resulting in a xenobiotic that has been transformed into a water-soluble compound that can be excreted through urine or bile.

Heat-treated foods and even MRPs structures formed therein have been demonstrated to modulate Phase I and Phase II enzymes in animal feeding trials. Wenzel et al. (2002) administered heat-treated casein that was selectively fortified with the MRP CML. After a feeding period of 10 days, Phase II glutathione *S* transferase (GST) activity in rat colonic enterocytes and kidneys increased by 64 and 86%, respectively, compared to control animals on a standard diet containing equivalent amounts of non-heated casein. Lindenmeier et al. (2002) first identified an MRP structure with strong Phase I and Phase II modulating activities that is formed in bread crust upon heat treatment. In accordance with the well-accepted fact of antioxidants being potent inducers of Phase II enzymes through cellular binding to the "antioxidant responsive element" (ARE), this compound was also characterized as the key antioxidant in bread crust.

Next to bread crust and malt, coffee brews were also studied for their effects on Phase II enzymes (Somoza et al. 2003). In this study, *N*-methylpyridinium iodide was identified as the key compound modulating Phase II GST. In vivo effects of a decaffeinated coffee beverage and *N*-methylpyridinium iodide were tested in a 15-day animal trial on rats. As a result, feeding of 4.5% coffee beverage resulted in an increase of Phase II GST and UDP-glucuronyl-transferase activity by 24 and 40%, respectively, compared to animals fed control diet. Animals on the

N-methylpyridinium diet showed an increase in liver Phase II UDP-glucuronyl-transferase of 65% compared to controls. Plasma total antioxidant capacity and plasma tocopherol were elevated in animals fed both coffee beverage and the *N*-methylpyridinium containing diet. Surprisingly, feeding of *N*-methylpyridinium also resulted in an increased total antioxidant capacity in the plasma, although, based on its chemical structure, this compound cannot act as a radical scavenging antioxidant. The mechanism by which *N*-methylpyridinium ions induce Phase II enzymes has recently identified by Böttler et al. (2010) who showed that this compound effectively induced the gene transcription and translocation of Nrf2, a major transcription factor leading to the expression of antioxidant and Phase II enzymes, such as GST. These results are in line with findings reported by Cavin et al. (2008) who demonstrated an induction of Nrf2-mediated cellular defence and alteration of detoxifying enzyme activities as mechanisms of chemoprotective effects of coffee in the liver of rats. However, controlled intervention trials are still needed to verify the contribution of *N*-methylpyridinium ions and potential other health beneficial compounds in coffee to the reduced risk for diseases such as various types of cancer which are associated with an endogenous load of reactive oxygen species and decreased activities of detoxification enzymes.

Final outlook

'Melanoidins: the forgotten class of food polymers'. This was the title initially proposed for the European COST Action 919 that started in 1999. Over the past 10 years, the scientific knowledge on this topic has been significantly increased. Despite the difficulties related to the lack of a defined structure and to the absence of common standards, more than 200 papers have been published on melanoidins in foods and the number of papers and citations increased year by year due to their technological, nutritional and physiological implications.

The reasons underlying this growing interest are related to the number of biological activities that melanoidins are able to exert. In addition, the availability of more powerful analytical techniques which have become available to investigate these structurally complex compounds and the development of procedures to assess specific biological activities such as the antioxidant (Serpen et al. 2007, Delgado-Andrade et al. 2005) or the antimicrobial activities (Rufián-Henares and Morales 2008b) favoured the growing research on food-derived melanoidins.

The results obtained up to now demonstrated that it is not possible to attribute the same chemical characteristics, biological properties and physiological activities to all kinds of melanoidins. Soluble versus insoluble as well as protein-rich versus polysaccharide-rich melanoidins display very different chemical structures and biological activities. Remarkably, a very recent paper of Paur et al. (2010) indicates growing evidence for the health benefits of coffee melanoidins, including even data that support the hypothesis that melanoidins are the main components determining the anti-inflammatory effect of coffee.

In most of the other cases, jeopardized and somehow contradictory evidence has been published. In particular, the findings which have been related to possible physiological relevance of melanoidin intake for human health are often very weak and not taking into account the low bioavailability of melanoidins. On the other hand, the awareness that the intake of melanoidins associated with the Western diet is probably in the order of grams per day necessarily leads to the need of a precise assessment of their contents in foods. This would offer the opportunity to control their quantitative formation or modulate their structure in many thermally processed foods. The ultimate challenge for the next future will be to optimize food processing technologies for a selective formation of health beneficial melanoidins.

References

Açar O, Gökmen V, Pellegrini N, Fogliano V (2009) Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. Eur J Food Sci Technol 229:961–969

Adams A, Borrelli RC, Fogliano V, De Kimpe N (2005) Comparative characterization of food melanoidins. J Agric Food Chem 53:4136–4142

Ames JM, Wynne A, Hofmann A, Plos S, Gibson GR (1999) The effect of a model melanoidin mixture on faecal bacterial populations in vitro. Br J Nutr 82:489–495

Anese M, Nicoli MC (2003) Antioxidant properties of ready-to-drink coffee brews. J Agric Food Chem 51:942–946

Babbs CF (1990) Free radicals and the etiology of colon cancer. Free Radic Biol Med 8:191–200

Bekedam EK, Schols HA, van Boekel MA, Smit G (2006) High molecular weight melanoidins from coffee brew. J Agric Food Chem 54:7658–7666

Bekedam EK, De Laat MP, Schols HA, Van Boekel MA, Smit G (2007) Arabinogalactan proteins are incorporated in negatively charged coffee brew melanoidins. J Agric Food Chem 55:761–768

Bekedam EK, Loots MJ, Schols HA, Van Boekel MA, Smit G (2008a) Roasting effects on formation mechanisms of coffee brew melanoidins. J Agric Food Chem 56:7138–7145

Bekedam EK, Roos E, Schols HA, Van Boekel MA, Smit G (2008b) Low molecular weight melanoidins in coffee brew. J Agric Food Chem 56:4060–4067

Bekedam EK, Schols HA, Cämmerer B, Kroh LW, van Boekel MA, Smit G (2008c) Electron spin resonance (ESR) studies on the formation of roasting-induced antioxidative structures in coffee brews at different degrees of roast. J Agric Food Chem 56:4597–4604

Bekedam EK, Schols HA, Van Boekel MA, Smit G (2008d) Incorporation of chlorogenic acids in coffee brew melanoidins. J Agric Food Chem 56:2055–2063

Bergmann R, Helling R, Heichert C, Scheunemann M, Mäding P, Wittrisch H, Johannsen B, Henle T (2001) Radio fluorination and positron emission tomography (PET) as a new approach to study the in vivo distribution and elimination of the advanced glycation endproducts *N* epsilon-carboxymethyllysine (CML) and *N* epsilon-carboxyethyllysine (CEL). Nahrung 45:182–188

Bichler J, Cavin C, Simic T, Chakraborty A, Ferk F, Hoelzl C, Schulte-Hermann R, Kundi M, Haidinger G, Angelis K, Knasmüller S (2007) Coffee consumption protects human lymphocytes against oxidative and 3-amino-1-methyl-5H-pyrido[4,3-b]indole acetate (Trp-P-2) induced DNA-damage: results of an experimental study with human volunteers. Food Chem Toxicol 45:1428–1436

Borrelli RC, Fogliano V (2005) Bread crust melanoidins as potential prebiotic ingredients. Mol Nutr Food Res 49:673–678

Borrelli RC, Fogliano V, Monti SM, Ames JM (2002) Characterisation of melanoidins from a glucose–glycine model system. Eur Food Res Technol 215:210–215

Borrelli RC, Mennella C, Barba F, Russo M, Russo GL, Krome K, Erbersdobler H, Faist V, Fogliano V (2003) Characterization of coloured compounds obtained by enzymatic extraction of bakery products. Food Chem Toxicol 41:1367–1374

Borrelli RC, Esposito F, Napolitano A, Ritieni A, Fogliano V (2004) Characterization of a new potential functional ingredient: coffee silverskin. J Agric Food Chem 52:1338–1343

Böttler U, Sommerfeld K, Volz N, Pahlke G, Teller N, Somoza V, Lang R, Hofmann T, Marko D (2010) Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. Mol Nutr Food Res (in press)

Brands CMJ, Wedzicha BL, van Boekel MAJS (2002) Quantification of melanoidin concentration in sugar-casein systems. J Agric Food Chem 50:1178–1183

Calligaris S, Manzocco L, Anese M, Nicoli MC (2004) Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. Int Dairy J 14:421–427

Cammerer B, Kroh LW (1995) Investigation of the influence of reaction conditions on the elementary composition of melanoidins. Food Chem 53:55–59

Cämmerer B, Jalyschko W, Kroh LW (2002) Intact carbohydrate structures as part of the melanoidin skeleton. J Agric Food Chem 50:2083–2087

Cavin C, Marin-Kuan M, Langouët S, Bezençon C, Guignard G, Verguet C, Piguet D, Holzhäuser D, Cornaz R, Schilter B (2008) Induction of Nrf2-mediated cellular defenses and alteration of phase I activities as mechanisms of chemoprotective effects of coffee in the liver. Food Chem Toxicol 46:1239–1248

COST (1998) Melanoidins in food and health: COST Action 919. European Union, Brussels, Belgium

D'Agostina A, Boschin G, Bacchini F, Arnoldi A (2004) Investigations on the high molecular weight foaming fractions of espresso coffee. J Agric Food Chem 52:7118–7125

Daglia M, Cuzzoni MT, Dacarro C (1994) Antibacterial activity of coffee. J Agric Food Chem 42:2270–2272

Daglia M, Tarsi R, Papetti A, Grisoli P, Dacarro C, Pruzzo C (2002) Antiadhesive effect of green and roasted coffee on *Streptococcus mutans*' adhesive properties on saliva-coated hydroxyapatite beads. J Agric Food Chem 50:1225–1229

Daglia M, Racchi M, Papetti A, Lanni C, Govoni S, Gazzani G (2004) In vitro and ex vivo antihydroxyl radical activity of green and roasted coffee. J Agric Food Chem 52:1700–1704

Delgado-Andrade C, Morales FJ (2005) Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. J Agric Food Chem 53:1403–1407

Delgado-Andrade C, Rufián-Henares JA, Morales FJ (2005) Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. J Agric Food Chem 53:7832–7836

Dell'Aquila C, Ames JM, Gibson GR, Wynne AG (2003) Fermentation of heated gluten systems by gut microflora. Eur Food Res Technol 217:382–386

Dittrich R, Dragonas C, Kannenkeril D, Hoffmann I, Mueller A, Beckmann MW, Pischetsrieder M (2009) A diet rich in Maillard reaction products protects LDL against copper induced oxidation ex vivo, a human intervention trial. Food Res Int 42:1315–1322

Einarsson H (1987) The effect pH and temperature on the antibacterial effect of Maillard reaction products. Lebensm Wiss Technol 20:51–55

Einarsson H, Snjgg BG, Eriksson C (1983) Inhibition of bacterial growth by Maillard reaction products. J Agric Food Chem 31:1043–1047

Erbersdobler HF, Gunsser I, Weber G (1970) Abbau von Fruktoselysin durch die Darmflora. Zbl Vet Med A 17:573–575

Erbersdobler HF, Purwing U, Bossen M, Trautwein EA (1986) Urinary excretion of fructoselysine in human volunteers and diabetic patients. In: Fujimaki M, Namiki N, Kato H (eds) Amino-carbonyl reactions in food and biological systems. Developments in food science 13. Elsevier, Tokyo, pp 503–508

Esposito F, Morisco F, Verde V, Ritieni A, Alezio A, Caporaso N, Fogliano V (2003) Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects. Aliment Pharmacol Ther 17:595–601

Faist V, Erbersdobler HF (2001) Metabolic transit and in vivo effects of melanoidins and precursors compounds deriving from the Maillard reaction. Ann Nutr Metab 45:1–12

Falcone PM, Giudici P (2008) Molecular size and molecular size distribution affecting traditional balsamic vinegar aging. J Agric Food Chem 56:7057–7066

Finot PA, Furniss DE (1989) Metabolic transit and toxicity of Maillard reaction products. Prog Clin Biol Res 304:343–358

Finot PA, Magnenat E (1981) Metabolic transit of early and advanced Maillard products. Prog Food Nutr Sci 5:193–207

Fogliano V, Monti SM, Musella T, Randazzo G, Ritieni A (1999) Formation of coloured Maillard reaction products in a gluten-glucose model system. Food Chem 66:293–299

Garsetti M, Pellegrini N, Baggio C, Brighenti F (2000) Antioxidant activity in human faeces. Br J Nutr 84:705–710

Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259–275

Giudici P, Gullo M, Solieri L, Falcone PM (2009) Chapter 4: technological and microbiological aspects of traditional balsamic vinegar and their influence on quality and sensorial properties. Adv Food Nutr Res 58:137–182

Gniechwitz D, Reichardt N, Meiss E, Ralph J, Steinhart H, Blaut M, Bunzel M (2008a) Characterization and fermentability of an ethanol soluble high molecular weight coffee fraction. J Agric Food Chem 56:5960–5969

Gniechwitz D, Reichardt N, Ralph J, Blaut M, Steinhart H, Bunzel M (2008b) Isolation and characterisation of a coffee melanoidin fraction. J Sci Food Agric 88:2153–2160

Gokmen V, Serpen A, Fogliano V (2009) Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. Trends Food Sci Technol 20:278–288

Gomyo T, Horikoshi M (1976) On the interaction of melanoidins with metallic ions. Agric Biol Chem 40:33–40

Goya L, Delgado-Andrade C, Rufián-Henares JA, Bravo L, Morales FJ (2007) Effect of coffee melanoidin on human hepatoma HepG2 cells. Protection against oxidative stress induced by *tert*-butylhydroperoxide. Mol Nutr Food Res 51:536–545

Hashiba H (1986) Oxidative browning of Amadori compounds. Color formation by iron with Maillard reaction products. In: Fujimaki M, Kato H, Namiki M (eds) Amino-carbonyl reactions in food and biological systems. Elsevier, Amsterdam, pp 155–164

Hidalgo FJ, Zamora R (2008) Food anoxia and the formation of either flavor or toxic compounds by amino acid degradation initiated by oxidized lipids. Ann N Y Acad Sci 1126:25–29

Hiramoto S, Itoh K, Shizuuchi S, Kawachi Y, Morishita Y, Nagase M, Suzuki Y, Nobuta Y, Sudou Y, Nakamura O, Kagaya I, Goshima H, Kodama Y, Icatro FC, Koizumi W, Saigenji K, Miura S, Sugiyama T, Kimura N (2004) Melanoidin, a food protein-derived advanced Maillard reaction product, suppresses *Helicobacter pylori* in vitro and in vivo. Helicobacter 9:429–435

Hodge JE (1953) Chemistry of browning reactions in models systems. J Agric Food Chem 1:928–943

Hofmann T (1998) Studies on the relationship between molecular weight and the color potency of fractions obtained by thermal treatment of glucose amino acid and glucose/protein solutions by using ultracentrifugation and color dilution techniques. J Agric Food Chem 46:3891–3895

Hofmann T, Schieberle P (2002) Chemical interactions between odoractive thiols and melanoidins involved in the aroma staling of coffee beverages. J Agric Food Chem 50:319–326

Hofmann T, Bors W, Stettmaier K (1999) Radical-assisted melanoidin formation during thermal processing of foods as well as under physiological conditions. J Agric Food Chem 47:391–396

Hofmann T, Czerny M, Calligaris S, Schieberle P (2001) Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. J Agric Food Chem 49:2382–2386

Homma S, Murata H, Terasawa N, Lee Y-S (1998) Characterisation of food melanoidins. In the Maillard reaction in foods and medicine. Royal Society of Chemistry, Cambridge, UK, p 412

Horikoshi M, Ohmura M, Gomyo T, Kuwabara Y, Ueda S (1981) Effects of browning products on the intestinal microflora of the rat. In Erikkson C (ed) Progress in food and nutrition science. Pergamon Press, Oxford, UK, pp 13–15, 223–228

Jägerstad M, Skog K, Solyakov A (2002) Effects of possible binding of potential human carcinogens in cooked foods to melanoidins. In: COST-919 proceedings, vol 1, pp 89–92

Jemmali MJ (1969) Influence of Maillard reaction products on some bacteria of intestinal flora. J Appl Bacteriol 32:151–154

Kato H, Hayase F (2002) An approach to estimate the chemical structure of melanoidins. Int Congress Ser 1245:3–7

Kuntcheva MJ, Obretenov TD (1996) Isolation and characterization of melanoidins from beer. Z Lebensm-Unters Forsch 202:238–243

Lanciotti R, Anese M, Sinigaglia M, Severini C, Massini R (1999) Effects of heated glucose–fructose–glutamic acid solutions on the growth of Bacillus stearothermophilus. Lebensm Wiss Technol 32:223–230

Lindenmeier M, Faist V, Hofmann T (2002) Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. J Agric Food Chem 50:6997–7006

López-Galilea I, De Peña MP, Cid C (2007) Correlation of selected constituents with the total antioxidant capacity of coffee beverages: influence of the brewing procedure. J Agric Food Chem 55:6110–6117

Maier HGE, Buttle H (1973) Isolation and characterization of brown roast compounds of coffee. 2. Z Lebensm-Unters Forsch A 150:331–334

Mattila T, Sandholm M (1989) Antibacterial effect of the glucose oxidase-glucose system on food-poisoning organisms. Int J Food Microbiol 8:165–174

Meshcheryakova S, Agienko K, Dostizh, Obi. Khim. (1987) Gigieny Obespech. Povysh. Kach. Moloch. Prod., Moskva, 95

Migo VP, del Rosario EJ, Matsumura M (1997) Flocculation of melanoidins induced by inorganic ions. J Ferment Bioeng 83:287–291

Milic BL, Grujic-Injac B, Piletic MV, Lajgic S, Kolarov LA (1975) Melanoidins and carbohydrates in roasted barley. J Agric Food Chem 23:960–963

Mok TA, Lynch SR, Cook JD (1983) Inhibition of food iron absorption by coffee. Am J Clin Nutr 37:416–420

Morales FJ (2002) Application of capillary zone electrophoresis to the study of food and food-model melanoidins. Food Chem 76:363–369

Morales FJ, Fernández-Fraguas MC, Jiménez-Pérez S (2005) Iron-binding ability of melanoidins from food and model systems. Food Chem 90:821–827

Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C (2002) Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 50:6211–6216

Nunes FM, Coimbra MA (2007) Melanoidins from coffee infusions. Fractionation, chemical characterization, and effect of the degree of roast. J Agric Food Chem 55:3967–3977

O'Brien J, Morrissey PA (1997) Metal ion complexation byproducts of the Maillard reaction. Food Chem 58:17–27

Obretenov T, Vernin G (1998) Melanoidins in the Maillard reaction. In: Contis ET, Ho C-T, Mussinan CJ, Parliment TH, Shahidi F, Spanier AM (eds) Food flavors: formation, analysis and packaging influences, vol 40. Elsevier Science, Amsterdam, pp 455–482

Obretenov TD, Kuntcheva MJ, Mantchev SC, Valkova GD (1991) Isolation and characterization of melanoidins from malt and malt roots. J Food Biochem 15:279–294

Obretenov TD, Ivanova SD, Kuntcheva MJ, Somov GT (1993) Melanoidin formation in cooked meat-products. J Agric Food Chem 41:653–656

Obretenov V, Demyttenaere J, Abbaspour-Tehrani K, Adams A, Kersiene M, De Kimpe N (2002) Flavor release in the presence of melanoidins prepared from L-(+)-ascorbic acid and amino acids. J Agric Food Chem 50:4244–4250

Oliviero T, Capuano E, Cammerer B, Fogliano V (2009) Influence of roasting on the antioxidant activity and HMF formation of a cocoa bean model systems. J Agric Food Chem 57:147–152

Ortega-Heras M, Gonzalez-Sanjosé ML (2009) Binding capacity of brown pigments present in special Spanish sweet wines. Food Sci Technol 42:1729–1737

Paur I, Balstad T, Blomhoff R (2010) Degree of roasting is the main determinant of the effects of coffee on NF-κB and EpRE. Free Radic Biol Med 48:1218–1227

Petracco M, Navarini L, Abatangelo A, Gombac V, D'Agnolo E, Zanetti F (1999) Isolation and characterisation of a foaming fraction from hot water extracts of roasted coffee. In: Proceedings of the 18th Colloquium of Science International Cafe, pp 95–105

Ponnampalam R, Mondy NI (2006) Effect of baking and frying on nutritive value of potatoes nitrogenous constituents. J Food Sci 48:1613–1616

Reichardt N, Gniechwitz D, Steinhart H, Bunzel M, Blaut M (2009) Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota. Mol Nutr Food Res 53(2):287–299

Rendleman JA (1987) Complexation of calcium by melanoidins and its role in determining bioavailability. J Food Sci 6:1699–1705

Rivero D, Perez-Magarino S, Gonzalez-Sanjose ML, Valls-Belles V, Codoner P, Muniz P (2005) Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and melanoidins. J Agric Food Chem 53:3637–3642

Rivero-Pérez MD, Pérez-Magariño S, González-SanJose ML (2002) Role of melanoidins in sweet wines. Anal Chim Acta 458:169–175

Rufián-Henares JA, De la Cueva SP (2009) Antimicrobial activity of coffee melanoidins—a study of their metal-chelating properties. J Agric Food Chem 57:432–438

Rufián-Henares JA, Morales FJ (2006) A new application of a commercial microtiter plate-based assay for assessing the antimicrobial activity of Maillard reaction products. Food Res Int 39:33–39

Rufián-Henares JA, Morales FJ (2007) Effect of in vitro enzymatic digestion on antioxidant activity of coffee melanoidins and fractions. J Agric Food Chem 55:10016–10021

Rufián-Henares JA, Morales FJ (2008a) Antimicrobial activity of melanoidins against *Escherichia coli* is mediated by a membrane damage mechanism. J Agric Food Chem 56:2357–2362

Rufián-Henares JA, Morales FJ (2008b) Microtiter plate-based assay for screening the specific antimicrobial activity of melanoidins. Food Chem 111:1069–1074

Sakurai H, Hashiba H, Okuhara A (1981) High molecular nitrogen-compounds in soy sauce. J Agric Chem Soc Jpn 55:7–14

Saura-Calixto F (1998) Antioxidant dietary fibre product: a new concept and a potential food ingredient. J Agric Food Chem 46:4303–4306

Serpen A, Capuano E, Fogliano V, Gokmen V (2007) A new procedure to measure the antioxidant activity of insoluble food components. J Agric Food Chem 55:7676–7681

Serpen A, Gökmen V, Pellegrini N, Fogliano V (2008) Direct measurement of total antioxidant capacity of cereal products. J Cereal Sci 48(2008):816–820

Smaniotto A, Bertazzo A, Comai S, Traldi P (2009) The role of peptides and proteins in melanoidin formation. J Mass Spectrom 44:410–418

Solyakov A, Skog K, Jegerstad M (2002) Binding of mutagenic/carcinogenic heterocyclic amines to MRPs under stimulated gastrointestinal conditions. In: Fogliano V, Henle T (eds) Melanoidins in food and health. COST Action 919, vol 3. European Communities, Luxembourg

Somoza V (2005) Five years of research on health risks and benefits of Maillard reaction products: an update. Mol Nutr Food Res 49:663–672

Somoza V, Lindenmeier M, Wenzel E, Frank O, Erbersdobler HF, Hofmann T (2003) Activity-guided identification of a chemopreventive compound in coffee beverage using in vitro and in vivo techniques. J Agric Food Chem 51:6861–6869

Somoza V, Wenzel E, Lindenmeier M, Grothe D, Erbersdobler HF, Hofmann T (2005) Influence of feeding malt, bread crust, and a pronylated protein on the activity of chemopreventive enzymes and antioxidative defense parameters in vivo. J Agric Food Chem 53:8176–8182

Somoza V, Wenzel E, Weiss C, Clawin-Rädecker I, Grübel N, Erbersdobler HF (2006) Dose-dependent utilisation of casein-linked lysinoalanine, *N*(epsilon)-fructoselysine and *N*(epsilon)-carboxymethyllysine in rats. Mol Nutr Food Res 50:833–841

Stadler RH, Varga N, Milo C, Schilter B, Vera FA, Welti DH (2002) Alkylpyridiniums. 2. Isolation and quantification in roasted and ground coffees. J Agric Food Chem 50:1200–1206

Stecchini ML, Giavedoni P, Sarais I, Lerici CR (1991) Effect of Maillard reaction products on the growth of selected food-poisoning micro-organism. Lett Appl Microbiol 13:93–96

Steginк LD, Freeman JB, Den Besten L, Filer LJ (1981) Maillard reaction products in parenteral nutrition. Prog Food Nutr Sci 5:265–268

Summa C, McCourt J, Cämmerer B, Fiala A, Probst M, Kun S, Anklam E, Wagner KH (2008) Radical scavenging activity, antibacterial and mutagenic effects of cocoa bean Maillard reaction products with degree of roasting. Mol Nutr Food Res 52:342–351

Tagliazucchi D, Verzelloni E, Conte A (2010) Effect of dietary melanoidins on lipid peroxidation during simulated gastric digestion: their possible role in the prevention of oxidative damage. J Agric Food Chem 58:2513–2519

Takenaka M, Sato N, Asakawa H, Wen X, Murate M, Homma S (2005) Characterization of a metal-chelating substance in coffee. Biosci Biotechnol Biochem 69:26–30

Tressl R, Wondrak GT, Kruger RP, Rewicki D (1998) New melanoidin-like Maillard polymers from 2-deoxypentoses. J Agric Food Chem 46:104–110

Viani R, Illy A (1995) Espresso coffee, the science of quality. Elsevier Academic Press, San Diego

Vitaglione P, Napolitano A, Fogliano V (2008) Cereal dietary fibre as natural functional ingredient to deliver phenolic compounds into the gut. Trends Food Sci Technol 19:451–463

Wang H, Jenner AM, Lee JCY, Shui G, Tang SY, Whiteman M, Wenk MR, Halliwell B (2007) The identification of antioxidants in dark soy sauce. Free Radic Res 41:479–488

Wenzel E, Tasto S, Erbersdobler HF, Faist V (2002) Effect of heat treated proteins on selected parameters of the biotransformation-system in the rat. Ann Nutr Metab 46:9–16

Wiame E, Delpierre G, Collard F, Van Schaftingen E (2002) Identification of a pathway for the utilization of the Amadori product fructoselysine in *Escherichia coli*. J Biol Chem 277:42523–42529

Wijewickreme AN, Kitts DD, Durance TD (1997) Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. J Agric Food Chem 45:4577–4583

Yen GC, Hsieh PP (1994) Possible mechanism of antimutagenic effect of Maillard reaction products prepared from xylose and lysine. J Agric Food Chem 42:133–137