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REVIEW ARTICLE

The gastro-intestinal tract as the major site of biological action of dietary melanoidins

Davide Tagliazucchi · Andrea Bellesia

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Abstract Emerging evidence from laboratory researches has highlighted the bioactivity of food melanoidins and melanoproteins. Whilst such studies have been carried out with different in vitro systems, information about melanoidins absorption and bio-availability are scarce. However, they are generally considered as poorly absorbable and bio-available compounds. Therefore, we present a review in which the gastro-intestinal tract is hypothesized to be the main site of action of food melanoidins and melanoproteins biological activity. We described recent data supporting this hypothesis both in vitro model systems and in vivo. Importantly, we focused this review only on the effect of melanoidins and melanoproteins extracted from food. Most of the studies had been carried out using water-soluble carbohydrate-based melanoidins isolated from different food sources (beer, barley coffee, coffee). In bakery products, melanoidins are protein-based structure (melanoproteins) which are largely insoluble in water. Dietary melanoidins and melanoproteins have been demonstrated to exert in vitro antioxidant and metal chelating ability in the gastro-intestinal tract reducing the formation of lipid hydroperoxides and advanced lipid oxidation end products during the digestion of meat. The reduction in the formation of these pro-atherogenic compounds has been shown to be followed by a decrease in their absorption in human volunteers. Food melanoidins have also shown in vitro anti-caries and prebiotic activities. We conclude by underlining the possible role of food melanoidins in the prevention of gastro-intestinal tract cancers. We hope this review will stimulate further research on food melanoidins and their biological activities in the gastro-intestinal tract.

Keywords Food melanoidins · Gastro-intestinal tract · Lipid hydroperoxides · Antioxidant activity · Cancer · Prebiotic

D. Tagliazucchi (✉) · A. Bellesia
Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2-Pad. Besta, 42100 Reggio Emilia, Italy
e-mail: davide.tagliazucchi@unimore.it

Introduction

Melanoidins are the final products of the Maillard reaction. Maillard reaction is a non-enzymatic browning reaction that occurs between the carbonyl group of reducing sugars and the amino group of amino acids, peptides or proteins during roasting, baking, cooking or ageing of foods and beverages. There are different steps in the Maillard reaction: (1) in the first step, the reaction between sugar and the amino group results in the formation of early stage compounds such as the Amadori-Heyns products; (2) in the second step, the Amadori-Heyns products undergo fragmentation resulting in the formation of low molecular weight, UV-absorbing compounds such as hydroxymethylfurfural, Strecker aldehydes, pyrazines or dicarbonyl compounds; (3) the final step involves cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensation reactions, which ultimately lead to the formation of the final reaction products, known as melanoidins (Hodge 1953).

Melanoidins are generically defined as brown-coloured, nitrogen-containing, high molecular weight compounds (Hodge 1953). Their chemical structure is still largely unknown despite their presence in a large range of thermally treated food products such as coffee, bread, biscuits, meat, barley coffee, beer, cocoa, and traditional balsamic

vinegar (Summa et al. 2008; Tagliazucchi et al. 2008, 2010; Fogliano and Morales 2011; Moreira et al. 2012).

Considering the high intake of melanoidins (Fogliano and Morales 2011), their biological activity and potential impact on human health are topics of great interest. Different in vitro biological activities have been attributed to melanoidins, namely, antioxidant, antimicrobial, prebiotic, anti-cancer, antihypertensive and anti-glycative activities (Rufián-Henares and Morales 2007, 2008a, b; Verzelloni et al. 2011; Borrelli and Fogliano 2005; Vitaglione et al. 2012).

Two major factors limit the actual physiological relevance of the biological activities of melanoidins. First, the limited knowledge of the structure of food melanoidins makes it difficult to identify the active principles responsible for the specific biological activity. Most studies have been carried out using the high molecular weight material (usually higher than 10 kDa) isolated from foods and beverages without further purification. Second, although melanoidins are consumed regularly as part of the daily human diet, they are generally considered as poorly absorbable and poorly bio-available compounds (Faist and Erbersdobler 2001).

For the reasons above stated, it is unlikely that dietary melanoidins could act as biologically active compounds in the bloodstream or organs. More important, most of the consumed melanoidins remain in the gastro-intestinal tract, therefore, it may be a key site for their antioxidant and biological action (Finot and Magnenat 1981; Rufián-Henares and Morales 2007; Delgado-Andrade 2014).

In this paper, a critical overview is presented about the possible impact of dietary melanoidins on the gastro-intestinal tract health and function. After a brief description of the chemical structure and the presence in foods of high molecular weight melanoidins, this review focuses on the hypothesis that the gastro-intestinal tract could be the site for the biological action of dietary melanoidins through a description of the most recent findings about the biological in vitro and in vivo effect of food melanoidins in the gastro-intestinal tract. Importantly, all of the studies discussed in this review concern exclusively the potential impact on the gastro-intestinal tract of melanoidins extracted from food and beverages.

Structural and chemical characteristics of food melanoidins and melanoproteins

The elucidation of the chemical and structural properties of melanoidins and melanoproteins is an important research area in food science and even though many efforts have been waged in the last years, the structural properties of food melanoidins are still largely unknown. The prominent difficulty in the study of the structure of food melanoidins is a consequence of their diversity and heterogeneity that reflect the complexity of the starting substrates, i.e. foods. Foods and beverages in fact contain numerous possible reagents which may be involved in the formation of melanoidins, such as amino acids, peptides, proteins, simple sugars and complex carbohydrates, polyphenols, etc. Therefore, distinct melanoidin populations, with different chemical (e.g. molecular weight, charge) and structural (depending on the nature of reactants) properties can be present in food (Table 1). Very recent review papers and research articles focused on this topic (Fogliano and Morales 2011; Wang et al. 2011; Moreira et al. 2012; Tagliazucchi and Verzelloni 2014; Pastoriza and Rufián-Henares 2014). In some foods such as coffee, cocoa, traditional balsamic vinegar, sweet wine and barley-derived beverages, most of the melanoidins are carbohydrate-based structures whereas in other foods (bakery foods) they are protein-based structures (melanoproteins). In addition to proteins/amino acids and carbohydrates, also other compounds can be incorporated into food melanoidins during their formation (Table 1).

Estimation of melanoidins and melanoproteins content in food and their dietary intake

Despite the fact that melanoidins are ubiquitous in our diet, there are sparse references in scientific literature about the estimation of melanoidin contents in different foodstuffs.

Different procedures have been applied for isolation and purification of food melanoidins. The method most widely accepted today takes advantage of their molecular weight and involves the use of different techniques such as dialysis or ultrafiltration with a molecular weight cutoff set at 3, 5 or 10 kDa. Once isolated, the melanoidin fractions are lyophilized and their contents expressed in weight on the basis of the dry matter of the initial food. This approach is limited in the sense that the high molecular weight material comprises other high molecular weight compounds (such as un-reacted polysaccharides, fibre or proteins), hampering a definitive conclusion about the estimation of the melanoidin content in food. However, to date, this is the best method used for the estimation of food melanoidins.

In coffee, the amount of melanoidin depends on the degree of roasting and coffee brew preparation. The more the coffee is roasted, the higher is the amount of melanoidins (Borrelli et al. 2002). Regarding the coffee preparation, the highest amount of melanoidins was found in soluble coffee (22.8 g in 100 g of coffee) whereas the amount of melanoidins in espresso, filtered and Italian preparation was found to be the same (7.2 g in 100 g coffee) (Fogliano and Morales 2011). As estimated by Fogliano and Morales (2011), the daily intake of coffee melanoidins ranged

Table 1 Structures and components of food melanoidins

Product	Structures	Components	References
Coffee	Carbohydrate-based	Galactomannans, arabino-galactan proteins, chlorogenic acids	Bekedam et al. (2008); Gniechwitz et al. (2008a); Nunes and Coimbra (2007); Moreira et al. (2012); Coelho et al. (2014)
	Non-carbohydrate-based	Phenolic/aromatic/olefinic structural units	Gniechwitz et al. (2008b)
Bakery products	Melanoproteins	Gluten polymers cross-linked to unknown low molecular weight, coloured Maillard reaction products	Borrelli et al. (2003); Rombouts et al. (2012)
Traditional balsamic vinegar	Carbohydrate-based	Glucose, fructose, proteins, phenolic moieties, Maillard reaction products	Verzelloni et al. (2010); Tagliazucchi and Verzelloni (2014)
	Non-carbohydrate-based	Hydroxymethylfurfural, Maillard reaction products	Verzelloni et al. (2010)
Barley coffee	Carbohydrate-based	Glucose, proteins, phenolic moieties, Maillard reaction products	Tagliazucchi et al. (2010); Tagliazucchi and Verzelloni (2014)
Dark beer	Carbohydrate-based	Glucose, proteins, phenolic moieties, Maillard reaction products	Rivero et al. (2005); Tagliazucchi et al. (2010); Tagliazucchi and Verzelloni (2014)
Cocoa	Carbohydrate-based	Polysaccharides, proteins, polyphenols (catechins)	Summa et al. (2008); Bellesia and Tagliazucchi (2014); Pastoriza and Rufián-Henares (2014)
Sweet wine	Carbohydrate-based	Polysaccharides, proteins, polyphenols	Pastoriza and Rufián-Henares (2014)
Nuts	No data	Fats	Acar et al. (2009)

between 0.5 and 2.0 g per day for moderate and heavy consumers, respectively.

A similar intake was calculated for bakery products by combining the mean quantity of consumption with the estimation of the melanoprotein content of the product (Fogliano and Morales 2011). In cereal products, melanoproteins are mainly present in bread crusts, while in dry biscuits, they are present in the whole product. The amount of melanoproteins in the bread crusts ranged from 14 to 30 g per 100 g of crust, depending on the type of bread but it decreased to 4.4 g per 100 g in the whole bread (Fogliano and Morales 2011; Pastoriza and Rufián-Henares 2014). Furthermore, the amount of melanoproteins found in dry biscuits ranged between 12 and 20 g per 100 g of whole product, whereas in breakfast cereals it was higher (25.5 g per 100 g).

For the calculation of the daily intake the authors referred to a study published by the Italian National Institute of Nutrition (INRAN) (Leclercq et al. 2009) which reported an average bread consumption among the Italian population of 103.3 g per day with a mean consumption among Italian bread consumers of 112.1 g per day. The same statistical research was made regarding the consumption of biscuits, defining an average intake of 13.8 g in Italian population with mean consumption of 27.3 g per day in consumers. Regarding breakfast cereals the average consumption was estimated at 1.5 and 14.1 g per day in Italian population and consumers, respectively. Combining the consumption data with the content of melanoproteins in bread, biscuits and breakfast cereals, the dietary intake of melanoproteins for bakery products can be estimated at around 6.5 g per day for average population and 12.3 g per day for consumers, respectively.

Regarding traditional balsamic vinegar (TBV), the high molecular weight melanoidins content ranged between 7.4 and 9.3 g per 100 g of TBV (Verzelloni et al. 2010). Considering the consumption of vinegar as a salad dressing in a teaspoon (15 g), the daily intake of melanoidins for consumers is in the range of 1–1.4 g per day.

There are different factors such as the temperature and time of fermentation process, type of grain used and colour which affect the melanoidin content of beer. Dark beer made using roasted malt or roasted barley showed a melanoidins content between 0.15 and 1.2 g/100 ml of beer (Rivero et al. 2005; Tagliazucchi and Verzelloni 2014). Pale beers contained less melanoidins, the concentration of which ranged between 0.06 and 0.34 g/100 ml of beer (Kuntcheva and Obretenov 1996; Rivero et al. 2005). Pilsner beer showed a greater melanoidins content ranging from 4 to 10.3 g/100 ml (Kuntcheva and Obretenov 1996; Pastoriza and Rufián-Henares 2014). According to the study of INRAN (Leclercq et al. 2009), we can estimate an average consumption of beer of 24.6 and of 148.7 mL

per day for Italian population and consumers, respectively. Considering a mixed consumption of different types of beer, the dietary intake of melanoidins for beer can be estimated around 1.3 g/day for average population and 7.7 g/day for consumers. For consumers of pilsner beer, the daily intake of melanoidins may reach amounts up to 15.3 g.

Sweet wine is another beverage rich in melanoidins which may contain between 11 and 17 g/100 mL of food melanoidins (Pastoriza and Rufián-Henares 2014). Considering an average sweet wine consumption in the Italian population of 2.3 mL (Leclercq et al. 2009) and an average melanoidins content for sweet wine of 14 g/100 mL, the estimated intake may be around 0.3 g per day. This value may increase up to 2.4 g per day in consumers (consumption of 17.4 mL of sweet wine; Leclercq et al. 2009).

Regarding cocoa, Bellesia and Tagliazucchi (2014) found a content of melanoidins in 100 % cocoa powder of 22 g/100 g. This value is in agreement with data reported by Pastoriza and Rufián-Henares (2014) who found a melanoidins content of 15 g/100 g in a chocolate sample containing 55 % of cocoa. Considering an average intake of chocolate/cocoa of 3.4 g per day in Italian population and 19 g per day in consumers (Leclercq et al. 2009), the intake of melanoidins from cocoa/chocolate products could be estimated between 0.6 and 3.5 g per day.

According to the studies of Fogliano and Morales (2011) and Pastoriza and Rufián-Henares (2014), a realistic estimation of melanoidins dietary intake for the general population would be close to 10–12 g per day, considering all the possible food sources (Table 2).

The gastro-intestinal tract as the major site for the biological activity of melanoidins

In this review we proposed that antioxidant activity and other protective effects of food melanoidins could occur within the gastro-intestinal tract itself. The rationale behind our hypothesis lies in two important observations about the dietary intake and metabolism of these compounds.

First, after the consumption of foods and beverages rich in melanoidins, such compounds can be present in the stomach and intestinal lumen at high concentrations, compatible with those shown in vitro biological effects. Second, although melanoidins are consumed regularly as part of the daily human diet, they are generally considered as poorly absorbable and poorly bio-available compounds (Faist and Erbersdobler 2001). The absorption of the melanoidins depends on their molecular weight and solubility (Finot and Magnenat 1981; Alamir et al. 2013; Nakano et al. 2013; Delgado-Andrade et al. 2013; Hellwig et al. 2014). The absorption of the low molecular weight and water-soluble melanoidins seems to be favoured. In rats

Table 2 Estimation of melanoidins content in food and their dietary intake

Product	Amount of melanoidins	Average daily intake (g per day per person)	Maximum daily intake (g per day per person)	References
Coffee	7.2–22.8 g/100 g depending on the coffee type	1	2	Fogliano and Morales (2011)
Cocoa/chocolate	15 g/100 g of chocolate (55 % cocoa); 22 g/100 g of 100 % cocoa powder	0.6	3.5	Pastoriza and Rufián-Henares (2014); Bellesia and Tagliazucchi (2014)
Bakery products	1.6–6.0 g/100 g depending on the bread type; 12–20 g/100 g for biscuit; 25.5 g/100 g for breakfast cereals	6.5	12.3	Fogliano and Morales (2011); Pastoriza and Rufián-Henares (2014)
Traditional balsamic vinegar	74–93 g/100 g	No data	1–1.4	Verzelloni et al. (2010); Tagliazucchi and Verzelloni (2014)
Barley coffee	1.44 g/100 g	No data	No data	Tagliazucchi and Verzelloni (2014)
Beer	0.06–10.3 g/100 mL depending on the beer type	1.3	7.7	Kuntcheva and Obretenov (1996); Rivero et al. (2005); Tagliazucchi et al. (2010); Pastoriza and Rufián-Henares (2014)
Sweet wine	11–17 g/100 mL depending on the sweet wine	0.3	2.4	Pastoriza and Rufián-Henares (2014)

70–90 % of orally ingested high molecular weight melanoidins (>10 kDa and prepared from amino acid/glucose and casein/glucose model systems) are excreted in faeces, and only 1–5 % absorbed and excreted in urine. Interestingly, the metabolic transit was similar for the melanoidins from both model systems (Finot and Magnenat 1981).

Bio-availability studies on isolated and chemically characterized Maillard reaction products (MRP), either free or protein-bound, showed that at least a part of them is absorbed during the intestinal transit (Delgado-Andrade et al. 2013; Förster et al. 2005). In a study with healthy adolescents aged 11–14 years, Delgado-Andrade et al. (2013) demonstrated that a MRP-high diet led to a higher *N*(ε)-carboxymethyllysine (CML) absorption and faecal excretion compared to a MRP-poor diet. Both absorption and faecal excretion of CML were highly influenced by dietary CML levels. However, they did not discriminate between free and bound CML. In rats fed with bread crust, faecal excretion of CML represented the major route of excretion (more than 30 %) (Roncero-Ramos et al. 2013c). More interestingly, CML-rich diet led to an accumulation of CML in rats cardiac tissue and tendons (Roncero-Ramos et al. 2014). Förster et al. (2005) found that pentosidine, was better absorbed when administered in a free form (coffee brew; about 60 % of absorption) than when ingested in a protein-bound form (bakery products; about 2 % of absorption).

The bio-availability seems to be related to the form in which the compounds are found in foods (free or protein-bound) and, in the case of the protein-bound form, to the ability of the gastro-intestinal proteases to release them from melanoproteins. In a simulated digestion experiment, carried out with MRP-modified casein (a model of melanoproteins), fructoselysine and CML were released from the MRP-casein complex whereas lysinoalanine was not so easily released and therefore less available for the absorption (Hellwig et al. 2014). An in vivo study (Somoza et al. 2006) performed in rats fed with MRP-modified casein substantially confirmed the in vitro results inferring that CML was more bio-available (about 30 % of urinary excretion) than fructoselysine and lysinoalanine.

Bio-availability data suggest that up to 30 % of the low molecular weight components of melanoidins or their intestinal degradation products can be absorbed, whereas a large proportion of the high molecular weight melanoidins are excreted in faeces (Delgado-Andrade 2014).

For the reasons above stated, it is unlikely that food melanoidins could act as biologically active compounds in the bloodstream or organs. More importantly, most of the consumed melanoidins remain in the gastro-intestinal tract, therefore, it may be a key site for their antioxidant and biological action (Finot and Magnenat 1981; Rufián-Henares and Morales 2007; Delgado-Andrade 2014). In addition, food high molecular weight melanoidins seem not to be degraded in the upper gastro-intestinal tract (Rufián-Henares and Morales 2007) and therefore enter the colon, where they and their products of bacterial fermentation can exert beneficial effects (Vitaglione et al. 2012).

The following sections of the paper review the studies performed to date on biological activities of food melanoidins in the gastro-intestinal tract (oral cavity, stomach, intestines and colon) or under gastro-intestinal in vitro conditions.

Most of the studies were carried out using water-soluble carbohydrate-based melanoidins isolated from different food sources such as beer, barley coffee and, especially, coffee. In other foods, especially bakery products, melanoidins are protein-based structures (melanoproteins) which are largely insoluble in water. Due to the difficulty to get this insoluble high molecular weight material, fewer studies have been carried out with melanoproteins. Most of these studies used an enzymatic approach to solubilised melanoproteins. In the subsequent sections the water solubility of the different populations of melanoidins used and the method used to solubilise melanoproteins are specified.

Antioxidant properties of food melanoidins in the gastro-intestinal tract

The most investigated biological activity of food melanoidins is the antioxidant activity (see Wang et al. 2011 for a recent review). Several studies have shown that melanoidins extracted from different foods possess radical scavenger activity, metal chelating ability and lipid peroxidation inhibitory activity under gastro-intestinal physiological conditions (Goya et al. 2007; Pastoriza and Rufián-Henares 2014; Tagliazucchi et al. 2010).

Rufián-Henares and Morales (2007) evaluated the impact of simulated gastro-pancreatic digestion on the radical scavenger ability of water-soluble coffee melanoidins isolated by ultrafiltration with a nominal cutoff of 10 kDa using several cell-free assays. They found that coffee melanoidins retained their radical scavenger ability even after the passage in the in vitro digestion system. Coffee high molecular weight melanoidins, therefore, seem not to be degraded in the first portion of the gastro-intestinal tract. A recent paper by Del Pino-García et al. (2012) showed that water-soluble high molecular weight melanoidins (>10 kDa) extracted from coffee and submitted to in vitro gastro-intestinal digestion exhibited high radical scavenger activity assayed with FRAP, ABTS, and DPPH methods. Also, the cold water-soluble high molecular weight fractions of coffee brews isolated by ultrafiltration and subjected to in vitro fermentation for 24 h with human faecal

bacteria still showed antioxidant properties (Reichardt et al. 2009).

Recently, a series of papers published by our group (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014) showed that water-soluble food melanoidins are efficient scavengers of the ABTS radical under gastric conditions (pH 2; 37 °C). Among the different foods, coffee melanoidins isolated by ultrafiltration (>10 kDa) exhibited six-fold higher radical scavenging activity than traditional balsamic vinegar melanoidins and eight- and 11-fold higher radical scavenging activity than barley coffee and dark beer melanoidins, respectively (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014). The radical scavenger activity of food melanoidins assayed under gastric conditions has been assigned to the presence of phenolic group in their structure (Tagliazucchi and Verzelloni 2014).

In vitro studies indicate, therefore, that food melanoidins retain radical scavenger activity along the entire gastro-intestinal tract suggesting a possible role of food melanoidins in the protection against the oxidative stress in this tract.

Antioxidant activity of water-soluble melanoidins isolated by ultrafiltration (>10 kDa) from coffee and water-insoluble melanoproteins isolated from biscuits (after enzymatic solubilisation) and subjected to consecutive gastro-pancreatic digestion was assayed on human hepatoma HepG2 cells (Goya et al. 2007; Martin et al. 2009). Coffee melanoidins completely abolished the cytoplasmatic formation of thiobarbituric acid reactive substances (TBA-RS) and also the depletion of intra-cellular reduced glutathione in the cells subjected to oxidative stress already at a concentration of 0.5 μg/mL. More interestingly, the pre-treatment of hepatoma cells with 5–10 μg/mL of digested coffee melanoidins completely avoided the tert-butylhydroperoxide (*t*-BOOH)-induced oxidative stress. The cells were exposed to the digested coffee melanoidins for 2 h, followed by washing, so that the extracellular presence of the coffee melanoidins was precluded when treatment with *t*-BOOH commenced. High molecular weight coffee melanoidins were found to be non-cytotoxic at concentrations up to 100 μg/mL. The pre-treatment of hepatoma cells with biscuit melanoproteins resulted in a protective effect against the oxidative stress induced by *t*-BOOH, albeit less effective than the coffee melanoidins.

Antioxidant properties of food melanoidins can result from their free radical scavenging activity but their ability to chelate transition metal ions also plays an important role. Dietary melanoidins are able to bind Ca^{2+}, Pb^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+} (Morales et al. 2012). The chelating ability of food melanoidins arises from their anionic nature which is strongly pH-dependent. Melanoidins exert a net negative electric charge at pH 5.0 and become more negative at higher pH values (Morales et al. 2012). High molecular weight water-soluble melanoidins (>10 kDa) extracted from different foods maintained the ability to chelate iron under gastric conditions (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014). Coffee melanoidins were more effective in chelate free iron ions respect to traditional balsamic vinegar, barley coffee and dark beer melanoidins (Tagliazucchi and Verzelloni 2014). Binding of ions in the gastro-intestinal tract may have negative health effects, possibly reducing the absorption and bio-availability of these ions. Mesías et al. (2009a) examined the effect of a diet rich in MRP on calcium bio-availability in healthy male adolescents. No significant changes in calcium bio-availability were observed between the MRP-rich and the MRP-poor diet. The same group tested on rats the effect of bread crust MRP on calcium, magnesium and phosphate bio-availability (Roncero-Ramos et al. 2012, 2013a, b). They concluded that the bio-availability of the tested ions was unmodified by consumption of bread crust or its isolate fractions. On the contrary, the bio-availability of iron was reduced by 2.7-fold in male adolescents who consumed a MRP-rich diet respect to the group fed with a MRP-poor diet (Mesías et al. 2009b). The reduction in iron bio-availability was mainly due to the effects found at the digestive level (Mesías et al. 2009b). Usually iron in the blood is bound to proteins to avoid the formation of free radicals. The excess of iron in the body causes several pathologies, because it becomes free from proteins and thus able to form reactive species and free radicals (Ronca et al. 2003). Melanoidins with their capacity to chelate iron lead to a decrease in its bio-availability possibly reducing the oxidative stress in the gastro-intestinal tract and in the body (Mesías et al. 2009b; Tagliazucchi et al. 2010; Verzelloni et al. 2010). In this regard, it has been shown that water-soluble high molecular weight melanoidins extracted from instant coffee and other foods are able to inhibit the formation of lipid hydroperoxide and advanced lipid oxidation end products (measured as TBA-RS) during simulated gastric digestion of turkey meat (Tagliazucchi et al. 2010; Verzelloni et al. 2010). Coffee melanoidins were the most effective respect to dark beer, barley coffee and traditional balsamic vinegar melanoidins and at a concentration of 3 mg/mL reversed the reaction and broke down hydroperoxides to a concentration lower than the initial value when digested with 300 g of turkey meat (Tagliazucchi et al. 2010). Recently, the anti-peroxidative activity of coffee melanoidins was demonstrated in an in vivo study (Sirota et al. 2013). The purpose of the study of Kanner and co-workers was to verify if the simultaneous consumption of 200 mL of coffee and 250 g of fast-food meat led to a reduction in the absorption of a specific advanced lipid oxidation end products (ALE), i.e. malondialdehyde (MDA). They measured the plasmatic level of MDA and found that

the consumption of roasted coffee during a meal of fast-food meat resulted, after 2 and 4 h, in the inhibition by 80 and 50 %, respectively, of post-prandial plasma MDA absorption. Although it was not possible to adequately identify the molecules (polyphenols and/or melanoidins) responsible for this effect, in vitro data (Tagliazucchi et al. 2010) strongly support the idea that high molecular weight coffee melanoidins are mainly responsible for the anti-peroxidative effect of coffee found in vivo.

Food melanoidins as dietary fibre and prebiotic

Dietary fibre is an important component of the human diet because of its high daily intake and its role in human intestinal health. Two recent researches within the European Prospective Investigation into Cancer and Nutrition (EPIC) study showed that dietary fibre intake was inversely associated with a lower risk of ischaemic heart disease and colon-rectal cancer (Crowe et al. 2012; Murphy et al. 2012).

Since melanoidins are formed during thermal treatment of food and contain amino acids/proteins, they cannot be exactly considered as dietary fibre. However, melanoidins and fibre appear to share some physical–chemical and physiological functions, and Silvan et al. (2010) proposed to redefine the concept of melanoidins in "maillardized fibre". In their paper they showed that during the roasting of coffee, about 45 % of soluble fibre turns into a maillardized structure. It was concluded that the content of coffee melanoidins includes part of the coffee dietary fibre and, viceversa, that coffee dietary fibre includes melanoidins.

Dietary fibre, maillardized fibre and melanoidins in coffee are fermented by human faecal microbiota resulting in the formation of acetate, propionate, and butyrate (Gniechwitz et al. 2008a; Reichardt et al. 2009). Maillardized insoluble dietary fibre has been detected also in bread as a complex between dietary fibre, proteins, Maillard products and polyphenols (Pérez-Jiménez et al. 2014).

Indeed, almost all of the chemically characterized food maillardized soluble and insoluble dietary fibre contain phenolic functional groups and can act as carriers of dietary antioxidants through the gastro-intestinal tract (Saura-Calixto 2011). The antioxidant bound to the dietary fibre can skip the absorption in the gut and can be released after fermentation of the carbohydrate moiety by colonic bacteria.

Most of these food maillardized dietary fibre carrying antioxidant compounds are poorly studied because they are not soluble in water or in the common organic solvents. Serpen et al. (2007) found that insoluble material in maillardized dietary fibre-rich foods (cereal-based foods) is able to exert a marked antioxidant activity. Pérez-Jiménez et al. (2014) described a significant increase in nonextractable antioxidants associated with insoluble dietary fibre in toasted bread and bread crust as compared with wheat flour.

The insoluble material in cereal-based food, which is mainly composed of proteins, polysaccharides, Maillard reaction products and polyphenols, may survive in the gastro-intestinal tract for a long time, scavenging free radicals that suggest a possible role of insoluble maillardized dietary fibre in the protection against the oxidative stress in the gastro-intestinal tract.

Food melanoidins may also act as prebiotic, able to modulate the bacterial colon population. Among the different groups present in human intestinal microbiome, *Bifidobacterium* spp and *Lactobacillus* spp are generally associated with a healthy intestinal condition, while *Clostridium* spp and *Bacteroides* spp are potentially dangerous. Bread crust melanoidins were fermented by colonic bacteria and able to selectively promote the increase in *Bifidobacterium* spp population in a static batch culture of faecal bacteria (Borrelli and Fogliano 2005). A similar effect was observed in two in vivo studies aimed to investigate the impact of coffee consumption on the gut bacterial population. A study carried out on human volunteers showed that the consumption of 3 cups per day of coffee during 3 weeks positively affected the population of *Bifidobacterium* spp (Jaquet et al. 2009). A more recent in vivo study was carried out on mice fed for 3 days with coffee (Nakayama and Oishi 2013). After coffee consumption, *Escherichia coli* and *Clostridium* spp counts significantly decreased in the proximal colon whereas the *Bifidobacterium* spp population increased in the same area.

Antimicrobial and anti-caries activity of food melanoidins

Several studies carried out in the last decade highlighted the antimicrobial activity of high molecular weight melanoidins extracted from different food sources such as coffee, beer, cocoa, and barley coffee as well as melanoproteins isolated from biscuits (Papetti et al. 2007; Summa et al. 2008; Rufián-Henares and Morales 2008a, b, 2009). Food melanoidins were active against both Gram-positive (such as *Streptococcus mutans*) and Gram-negative (such as *Escherichia coli*) bacteria, to different extents depending on the type of bacteria and food melanoidins.

Regarding the possible relevance for the gastro-intestinal tract, particular emphasis should be given to the anti-cariogenic potential of food melanoidins. The most important pathogenic bacteria involved in the development of dental caries is the Gram-positive bacteria *Streptococcus mutans*. Its cariogenic potential is in part related to its ability to adhere to the tooth surface and form a bio-film

(Senadheera and Cvitkovitch 2008). In a first study, Daglia et al. (2002) reported the anti-adhesive effect of green and roasted coffee. Both coffees tested were able to inhibit the adsorption of *S. mutans* to saliva-coated hydroxyapatite. More interesting, roasted coffee samples were significantly more active than the corresponding green coffee samples. In a subsequent work by the same group, water-soluble coffee melanoidins were unequivocally identified as in vitro anti-cariogenic compounds in roasted coffee (Stauder et al. 2010). The whole high molecular weight fraction of roasted coffee (>3.5 kDa) at concentration of 6 mg/mL showed potent adhesion inhibitory activity (91 % of inhibition), antimicrobial activity and inhibitory activity against *S. mutans* bio-film formation (100 % of inhibition). The high molecular weight coffee fraction was subsequently fractionated using gel filtration chromatography. The obtained melanoidin fractions were active against *S. mutans* adhesion and bio-film formation.

Barley coffee melanoidins have been also tested for their anti-cariogenic activity in vitro (Papetti et al. 2007). High molecular weight barley coffee fraction (>1 kDa and consisting of water-soluble melanoidins) displayed anti-adhesive and anti-bio-film properties. The high molecular weight fraction of barley coffee was further fractionated using a combination of dialysis and gel filtration chromatography. The most active fraction was found to consist of a single brown component with molecular weight higher than 1000 kDa.

Helicobacter pylori is the primary etiological agent in the development of peptic ulcers and gastric cancer (Lamb and Chen 2013). Extracellular urease plays a pivotal role for the host colonization because of its involvement in the processes of the adhesion to the gastric mucosa by *H. pylori* (Icatlo et al. 2003). Hiramoto et al. (2004) showed that a variety of food protein-derived melanoidins (from casein and muffin crust, isolated by ultrafiltration with a cutoff of 100 kDa) were able to strongly inhibit the in vitro urease-gastric mucin adhesion. The effect was observed also in vivo. In particular, the casein-derived high molecular weight melanoidins were able to suppress colonization of *H. pylori* in mice and humans.

A variety of high molecular weight food melanoidins were also able to exert antimicrobial activity against *Escherichia coli*, a Gram-negative bacteria which is non-desirable in a large presence in the gut microflora and can cause severe diarrhoea.

Rufián-Henares and Morales (2008b) tested water-soluble coffee (extracted by ultrafiltration with a cutoff of 10 kDa) melanoidins and water-insoluble biscuit (enzymatically solubilized and extracted by ultrafiltration with a cutoff of 10 kDa) melanoproteins for their antimicrobial activity against *E. coli*. The antimicrobial activity was expressed as MIC (minimum inhibitory concentration), defined as the lowest concentration of melanoidin fractions not producing any detected cell growth (Rufián-Henares and Morales 2008b). Biscuit melanoproteins demonstrated higher antimicrobial activity (MIC value 7.5 mg/mL) than high molecular weight coffee melanoidins (MIC value 10 mg/mL). In another study (Rufián-Henares and Morales 2008a), the same authors showed that coffee melanoidins had higher antimicrobial activity than beer melanoidins. Summa et al. (2008) reported that all the high molecular weight cocoa fractions (>30, 30–10, and 10–5 kDa) tested were effective in reducing the growth of *Escherichia coli* and *Enterobacter cloacae*.

The possible role of food melanoidins in the protection of gastro-intestinal tract cancers

Gastro-intestinal tract tumours are one of the most common forms of neo-plastic diseases affecting humans. In particular colon-rectal cancer represents the second most frequent cause of cancer death in the USA (Edwards et al. 2010). The incidence of gastro-intestinal cancers varies greatly depending on the geographical area. They are common in most Western countries but are rare in developing countries, with lower rates in middle- and high-poverty countries (Center et al. 2009). Indeed, the colorectal cancer incidence rates continue to increase in economically transitioning countries (Center et al. 2009). In part, these variations may indicate that the major causes for gastro-intestinal cancers are dietary habits and lifestyle factors (such as lack of physical activity and smoking) (Slattery et al. 1999). Excessive intake of protein, fat, and alcohol increases the risk of gastro-intestinal cancers (Willett 1999). Diet is not only a risk factor for the onset of gastro-intestinal cancers but can also be preventive. Some foods, such as vegetables, beverages, and fruit have been shown to induce a chemoprotective action on the gastro-intestinal tract (Willett 1999).

The most studied anti-cancer activity of food high molecular weight melanoidins involved their ability to modulate the activity of detoxifying enzymes in colon carcinoma cells model system (usually Caco-2). The detoxification from xenobiotics occurs in two phases which are called Phase I (functional group modification) and Phase II (conjugation). The most important enzymes involved in Phase I reactions are the cytochrome P450 (CYP450) isoenzymes which use oxygen and NADH to promote the addition of a reactive hydroxyl group to the substrates. The result of this reaction is the generation of reactive molecules, which may be more reactive than the parent molecule. The Phase II detoxification reactions generally follow the Phase I reaction. Xenobiotics and carcinogen activated by the Phase I reaction are further metabolized by Phase

II conjugation reactions. The result is the conjugation of the reactive molecules with a polar group to produce more water-soluble and easy to excrete compounds. The balance between the activity of Phase I and Phase II enzymes may play a paramount role in the increased risk for different type of cancers. For example, human deficiencies in Phase II enzyme activity, specifically glutathione-*S*-transferase (GST), have been identified and associated with increased risk for colon cancer (Wilkinson and Clapper 1997).

The first melanoidin-rich food studied for its potential chemopreventive activity was bread crust. Lindenmeier et al. (2002) fractionated with different solvents the brown crust isolated from bread and tested the different fractions for their chemopreventive potential. The intensively brown ethanolic crust fraction (mainly composed of water-insoluble melanoproteins) was the most effective in inducing a significantly elevated GST activity and a decreased Phase I (NADPH-cytochrome *c* reductase) activity in Caco-2 cells. The compound responsible for this effect was identified as protein-bound pyrrolinone reductonyl-lysine (abbreviated as pronyl-lysine) structure (Lindenmeier et al. 2002). Next, Borrelli et al. (2003) investigated the Phases I and II modulating activity of food water-insoluble melanoproteins enzymatically extracted from biscuits. The exposure of Caco-2 cells to the biscuit extract resulted in a decreased activity of both NADPH-cytochrome *c* reductase and GST.

In vivo effects of malt, bread crust, and pronylated protein were tested in a 15-day animal trial on rats (Somoza et al. 2005). As a result, feeding of 5 % bread crust resulted in a 18 % elevated activity of GST in the kidneys whereas the administration of pronyl bovine serum albumin (BSA) caused an increase of 27 % of liver UDP-glucuronyl transferase. In two additional in vivo studies, the chemopreventive potential of pronyl-lysine extracted from bread crust was assayed using rats treated with the carcinogen 1,2-dimethyl hydrazine. Pronyl-lysine was able to reduce the total aberrant crypt foci formation, total number of dysplastic foci, and cell proliferation in the colon, suggesting that pronyl-lysine suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis effectively (Selvam et al. 2009a). The anti-cancer effect of pronyl-lysine in colon has been shown to be related to its ability to reduce oxidative stress during colon carcinogenesis induced by 1,2-dimethylhydrazine (Selvam et al. 2009b).

Matrix metalloproteases (MMPs) are a class of zinc-containing endo-peptidases which are over-expressed in human colorectal cancer (Zucker and Vacirca 2004). They are involved in the degradation of extracellular matrix during the metastatic process. Inhibition of MMPs synthesis and activity could be an interesting approach for colon cancer therapy together with chemotherapeutic drugs (Zucker et al. 2000). The potential inhibitory activity of coffee melanoidins against recombinant human MMPs was assayed by De Marco et al. (2011). Water-soluble high molecular weight coffee melanoidins (extracted by ultrafiltration at 10 kDa cutoff) were able to inhibit MMPs with IC_{50} value between 0.2 and 0.7 mg/mL. Considering that the colon accumulates its content over at least 24 h in a maximum volume of 2 l, and that the daily intake of coffee melanoidins ranges between 0.5 and 2.0 g (Fogliano and Morales 2011), it is possible to calculate a hypothetical concentration of coffee melanoidins in the colon between 0.25 and 1 mg/mL, which are values comparable to the IC_{50} for MMPs inhibition.

POTEX is a potato fibre preparation broadly used in the meat and bakery industry (Langner et al. 2011). Normally, POTEX-containing foods are thermally treated before consumption. This results in the formation of water-soluble high molecular weight melanoidins from POTEX polysaccharides and proteins (Langner et al. 2011). POTEX water-soluble melanoidins (isolated by ultrafiltration >10 kDa) revealed a dose-dependent antiproliferative activity against LS180 colon cancer cell line without showing any cytotoxic effect in normal colon epithelial cell line (Langner et al. 2011, 2013). POTEX melanoidins act through a reduction in the level of cell cycle promoters cyclin D1 and cyclin-dependent kinases and an increase in the level of several cell cycle inhibitors (such as p21, p27, and p53) through ERK1/2 signalling hyper-activation.

Several epidemiological studies described the possible association between coffee consumption and the development of colorectal cancer. Although solid conclusions on the association between coffee consumption and risk of colon cancer have not been obtained yet, some recent meta-analyses of prospective cohort studies seem to suggest the existence of an inverse relationship between coffee consumption and colorectal cancer risk. In a meta-analysis of 12 prospective cohort studies, Je and co-workers (2009) concluded that coffee drinkers do not have a decreased risk of colorectal, colon or rectal cancer. Interestingly, they found a marginally lower incidence of colon cancer in women who drank more than 4 cups of coffee per day. In a subsequent meta-analysis carried out on 15 prospective cohort studies, Yu et al. (2011) suggested that coffee consumption has an inverse association with some type of cancers including colon cancer. In a very recent meta-analysis of 16 prospective cohort studies, Li and colleagues (2013) found a slight inverse association between coffee consumption and colorectal and colon cancer.

Given this consideration, it is surprising that literature is lacking in investigations focused on the direct effects of coffee bioactive compounds (including melanoidins) on colon cancer. Recently, Vitaglione et al. (2012) reviewed the possible mechanisms by which coffee bioactives (chlorogenic acids and melanoidins) may influence the risk of colorectal cancer development.

Table 3 Summary of the possible mechanism correlating melanoidins intake to the reduction of gastro-intestinal cancer risk

Biological activity	Biological effect	Food melanoidins	References
Enzyme modulating activity	Reduction of carcinogen activation and reduction in tumour progression and metastasis	Coffee, malt, bread crust, pronyl-lysine	Lindenmeier et al. (2002); Borrelli et al. (2003); De Marco et al. (2011)
Antiproliferative activity	Reduction in tumour growth and reduction of the total number of crypts in rats	POTEX, pronyl-lysine	Langner et al. (2011); Langner et al. (2013); Selvam et al. (2009a)
Gastric and colon motility	Increase in carcinogen elimination	Coffee	Vitaglione et al. (2012); Argirova et al. (2013)
Prebiotic activity	Amelioration of insulin sensitivity and body weight loss	Coffee	Vitaglione et al. (2012)
Antioxidant activity	Reduction of oxidative stress in the colon, inhibition of DNA oxidative damage and inflammation	Coffee and many others food melanoidins	Tagliazucchi et al. (2010); Del Pino et al. (2012); Vitaglione et al. (2012); Sauer et al. (2013)
Chelating ability	Reduction in carcinogen formation (*N*-nitroso compound) and reduction in cytotoxicity	Coffee, barley coffee, dark beer, and traditional balsamic vinegar melanoidins	Tagliazucchi et al. (2010); Verzelloni et al. (2010); Tagliazucchi and Verzelloni (2014)

Three possible pathways correlating coffee intake to the reduction of colorectal cancer risk were suggested as follows: (1) increase in colon motility which results in an increased carcinogen elimination rate (coffee dietary fibre and melanoidins); (2) modulation of gut microbiota which could result in an amelioration of insulin sensitivity and body weight loss, reducing colon cancer risk (coffee dietary fibre and melanoidins); and (3) reduction in the inflammation in colon mucosa by coffee antioxidants resulting in a reduced colon cancer risk (melanoidins). Although the hypothesis is speculative and not investigated till now, their conclusions should be considered the starting point to study the possible ability of coffee melanoidins/dietary fibre to positively influence the colon function.

Very recently, Argirova and colleagues (2013), demonstrated ex vivo the ability of coffee water-soluble melanoidins (isolated by ultrafiltration, cutoff 5 kDa) to induce contractions in gastric smooth muscle. Coffee melanoidins provoked a depolarization of smooth muscle membranes which resulted in an increased afflux of Ca^{2+} into the cell. Coffee melanoidins were able to induce the contraction of gastric smooth muscle cells by interacting with muscarinic acetylcholine receptors.

In addition to direct antioxidant activity, coffee melanoidins may also exert indirect antioxidant effects. Recent evidence suggests that some coffee components formed during roasting are able to induce the transcription factor nuclear factor-erythroid-2-related factor (Nrf2) in macrophages, Caco-2 cells and intact human gut tissue (Sauer et al. 2013). After translocation into the nucleus, Nrf2 binds to the antioxidant response element (ARE) inducing the expression of some enzymes (such as glutathione synthetase, catalase, thioredoxin, Phase II enzymes, etc.) involved in the cellular antioxidant response to the oxidative stress (Li et al. 2008). Whether or not coffee melanoidins are responsible for this effect is still not known. Indeed, the activation of Nrf2 could result in an attenuation of NFkB activation, which has been associated with inflammation, cellular oxidative stress and neoplasia in colon (Li et al. 2008).

An additional mechanism which could be related to the anti-cancer activity of melanoidins in the gastro-intestinal tract is their heme-binding ability. Heme can act as a catalyst for oxidative damage and can initiate colorectal cancer (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014). Dietary water-soluble melanoidins were able to bind heme under gastro-intestinal conditions (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014). Coffee melanoidins had greater affinity towards heme in comparison to barley coffee, dark beer, and traditional balsamic vinegar melanoidins (Tagliazucchi and Verzelloni 2014). Melanoidins may

act in the gastro-intestinal tract as "sponges" capable of sequestering the heme groups released during the digestion of meat and delivering them to the faeces where they are then excreted.

Table 3 represents a summary of the possible mechanisms of melanoidins protection towards a reduction of gastro-intestinal cancer risk.

Conclusion

In recent years, an increasing number of studies have been published regarding the possible effects of melanoidins in the gastro-intestinal tract. Due to their low bio-availability, it is unlikely that melanoidins can exert their protective effects at the systemic level. More plausibly, melanoidins can act at gastro-intestinal level where they reach high concentration following dietary intake. Most of the studies have been carried out in vitro and suffer some limitations concerning mainly the lack of knowledge about the structure of melanoidins. It is becoming increasingly clear that in foods a single type of melanoidin does not exist but different melanoidin populations co-exist within a single sample. Indeed, the results obtained until now have demonstrated that different melanoidin populations behave differently and have different biological properties and physiological activities. For this reason, an important future effort must be made to isolate and purify the various structures within a food.

Some of the effects attributed to melanoidins at gastro-intestinal level were also found in vivo. For example, in the stomach they act as antioxidants and metal chelators, inhibiting the peroxidation of meat lipids and decreasing the synthesis of hydroperoxides and ALEs. The reduction in the formation of these pro-atherogenic compounds has been shown to be followed by a decrease in their absorption in human volunteers. The ability of melanoidins to inhibit lipid peroxidation may contribute to their health benefits, since dietary oxidized lipid and ALEs are involved in the development of atherosclerosis and other diseases. Also, the metal chelating ability of melanoidins in healthy humans and rats has been studied. MRP-rich diet did not modify the bio-availability of calcium, magnesium and phosphate, whereas the bio-availability of iron was reduced by 2.7-fold in male adolescents.

Last but not least, it is necessary that future studies are designed to demonstrate the anti-cancer activities of food melanoidins with special emphasis given to their prebiotic and antioxidant effects.

Conflict of interest The authors declare that they have no conflict of interest.

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