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The gastro-intestinal tract as the major site of biological action of dietary melanoidins / Tagliazucchi, Davide; Bellesia, Andrea. - In: AMINO ACIDS. - ISSN 0939-4451. - STAMPA. - 47:6(2015), pp. 1077-1089. [10.1007/s00726-015-1951-z]

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09/01/2026 11:51

# **The gastrointestinal tract as the major site of biological action of dietary melanoidins**

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## Abstract

Emerging evidence from laboratory researches have 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, 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.

26    **Keywords:** food melanoidins, gastro-intestinal tract, lipid hydroperoxides, antioxidant  
27    activity, cancer, prebiotic.

28

## 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-Heynes products; (2) in the second step the Amadori-Heynes 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; Tagliazucchi et al. 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 is a topic 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; Rufián-Henares and Morales 2008a 2009; 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. Secondly, 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**

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

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

116 In coffee, the amount of melanoidin depends on the degree of roasting and coffee  
117 brew preparation. The more the coffee is roasted, the higher is the amount of  
118 melanoidins (Borrelli et al. 2002). Regarding the coffee preparation, the highest  
119 amount of melanoidins was found in soluble coffee (22.8 g in 100 g of coffee)  
120 whereas the amount of melanoidins in espresso, filtered and Italian preparation was  
121 found to be the same (7.2 g in 100 g coffee) (Fogliano and Morales 2011). As  
122 estimated by Fogliano and Morales (2011), the daily intake of coffee melanoidins  
123 ranged between 0.5 to 2.0 g per day for moderate and heavy consumers, respectively.

124 A similar intake was calculated for bakery products by combining the mean quantity  
125 of consumption with the estimation of the melanoprotein content of the product  
126 (Fogliano and Morales 2011). In cereal products, melanoproteins are mainly present  
127 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) (Leclercg 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 to 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

153 beers contained less melanoidins, the concentration of which ranged between 0.06 and  
154 0.34 g/100 ml of beer (Kuntcheva and Obretenov 1996; Rivero et al. 2005). Pilsner  
155 beer showed a greater melanoidins content ranging from 4 to 10.3 g/100 ml  
156 (Kuntcheva and Obretenov 1996; Pastoriza and Rufián-Henares 2014). According to  
157 the study of INRAN (Leclercg et al. 2009), we can estimate an average consumption  
158 of beer of 24.6 mL per day and of 148.7 mL per day for Italian population and  
159 consumers, respectively. Considering a mixed consumption of different types of beer,  
160 the dietary intake of melanoidins for beer can be estimated around 1.3 g/day for  
161 average population and 7.7 g/day for consumers. For consumers of pilsner beer, the  
162 daily intake of melanoidins may reach amounts up to 15.3 g.

163 Sweet wine is another beverage rich in melanoidins which may contain between 11  
164 and 17 g/100 mL of food melanoidins (Pastoriza and Rufián-Henares 2014).  
165 Considering an average sweet wine consumption in the Italian population of 2.3 mL  
166 (Leclercg et al. 2009) and an average melanoidins content for sweet wine of 14 g/100  
167 mL, the estimated intake may be around 0.3 g per day. This value may increase upto  
168 2.4 g per day in consumers (consumption of 17.4 mL of sweet wine; Leclercg et al.  
169 2009).

170 Regarding cocoa, Bellesia and Tagliazucchi (2014) found a content of melanoidins in  
171 100% cocoa powder of 22 g/100 g. This value is in agreement with data reported by  
172 Pastoriza and Rufián-Henares (2014) who found a melanoidins content of 15 g/100 g  
173 in a chocolate sample containing 55% of cocoa. Considering an average intake of  
174 chocolate/cocoa of 3.4 g per day in Italian population and 19 g per day in consumers  
175 (Leclercg et al. 2009), the intake of melanoidins from cocoa/chocolate products could  
176 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.

Firstly, 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. Secondly, 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 70 to 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 to 5% absorbed and excreted in urine. Interestingly, the metabolic transit was similar for the melanoidins from both model systems (Finot and Magnenat 1981).

202 Bio-availability studies on isolated and chemically characterized Maillard reaction  
203 products (MRP), either free or protein-bound, showed that at least a part of them is  
204 absorbed during the intestinal transit (Delgado-Andrade et al. 2013; Forster et al.  
205 2005). In a study with healthy adolescents aged 11–14 years, Delgado-Andrade et al.  
206 (2013) demonstrated that a MRP-high diet led to a higher N(ε)-carboxymethyllysine  
207 (CML) absorption and faecal excretion compared to a MRP-poor diet. Both  
208 absorption and faecal excretion of CML were highly influenced by dietary CML  
209 levels. However, they did not discriminate between free or bound CML. In rats fed  
210 with bread crust, faecal excretion of CML represented the major route of excretion  
211 (more than 30%) (Roncero-Ramos et al. 2013c). More interestingly, CML-rich diet  
212 led to an accumulation of CML in rats cardiac tissue and tendons (Roncero-Ramos et  
213 al. 2014). Förster et al. (2005) found that pentosidine, was better absorbed when  
214 administered in a free form (coffee brew; about 60% of absorption) than when  
215 ingested in a protein-bound form (bakery products; about 2% of absorption).  
216 The bio-availability seems to be related to the form in which the compounds are found  
217 in foods (free or protein-bound) and, in the case of the protein-bound form, to the  
218 ability of the gastro-intestinal proteases to release them from melanoproteins. In a  
219 simulated digestion experiment, carried out with MRP-modified casein (a model of  
220 melanoproteins), fructoselysine and CML were released from the MRP-casein  
221 complex whereas lysinoalanine was not so easily released and therefore less available  
222 for the absorption (Hellwig et al. 2014). An *in vivo* study (Somoza et al. 2006)  
223 performed in rats fed with MRP-modified casein substantially confirmed the *in vitro*  
224 results inferring that CML was more bio-available (about 30% of urinary excretion)  
225 than fructoselysine and lysinoalanine.

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

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

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

242 Most of the studies were carried out using water-soluble carbohydrate-based  
243 melanoidins isolated from different food sources such as beer, barley coffee and,  
244 especially, coffee. In other foods, especially bakery products, melanoidins are protein-  
245 based structures (melanoproteins) which are largely insoluble in water. Due to the  
246 difficulty to get this insoluble high molecular weight material, less studies have been  
247 carried out with melanoproteins. Most of these studies used an enzymatic approach to  
248 solubilised melanoproteins. In the subsequent sections the water solubility of the  
249 different populations of melanoidins used and the method used to solubilise  
250 melanoproteins is 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 cut-off 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 24h 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

276 conditions (pH 2; 37°C). Among the different foods, coffee melanoidins isolated by  
277 ultrafiltration (> 10 kDa) exhibited six-fold higher radical scavenging activity than  
278 traditional balsamic vinegar melanoidins and eight- and eleven-fold higher radical  
279 scavenging activity than barley coffee and dark beer melanoidins, respectively  
280 (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014).  
281 The radical scavenger activity of food melanoidins assayed under gastric conditions  
282 has been assigned to the presence of phenolic group in their structure (Tagliazucchi  
283 and Verzelloni 2014).  
284 *In vitro* studies indicate, therefore, that food melanoidins retain radical scavenger  
285 activity along the entire gastro-intestinal tract suggesting a possible role of food  
286 melanoidins in the protection against the oxidative stress in this tract.  
287 Antioxidant activity of water-soluble melanoidins isolated by ultrafiltration (> 10  
288 kDa) from coffee and water-insoluble melanoproteins isolated from biscuits (after  
289 enzymatic solubilisation) and subjected to consecutive gastro-pancreatic digestion  
290 was assayed on human hepatoma HepG2 cells (Goya et al. 2007; Martin et al. 2009).  
291 Coffee melanoidins completely abolished the cytoplasmatic formation of  
292 thiobarbituric acid reactive substances (TBA-RS) and also the depletion of intra-  
293 cellular reduced glutathione in the cells subjected to oxidative stress already at a  
294 concentration of 0.5 µg/mL. More interestingly, the pre-treatment of hepatoma cells  
295 with 5-10 µg/mL of digested coffee melanoidins completely avoided the *tert*-  
296 butylhydroperoxide (*t*-BOOH)-induced oxidative stress. The cells were exposed to the  
297 digested coffee melanoidins for 2 hours, followed by washing, so that the extra-  
298 cellular presence of the coffee melanoidins was precluded when treatment with *t*-  
299 BOOH commenced. High molecular weight coffee melanoidins were found to be non-  
300 cytotoxic at concentrations upto 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  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Fe}^{2+}$  (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; Roncero-Ramos et al. 2013a; Roncero-Ramos et al. 2013b). 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



326 at the digestive level (Mesías et al. 2009b). Usually iron in the blood is bound to  
327 proteins to avoid the formation of free radicals. The excess of iron in the body causes  
328 several pathologies, because it becomes free from proteins and thus able to form  
329 reactive species and free radicals (Ronca et al. 2003). Melanoidins with their capacity  
330 to chelate iron, lead to a decrease in its bio-availability possibly reducing the  
331 oxidative stress in the gastro-intestinal tract and in the body (Mesías et al. 2009b;  
332 Tagliazucchi et al. 2010; Verzelloni et al. 2010). In this regard, it has been shown that  
333 water-soluble high molecular weight melanoidins extracted from instant coffee and  
334 other foods are able to inhibit the formation of lipid hydroperoxide and advanced lipid  
335 oxidation endproducts (measured as TBA-RS) during simulated gastric digestion of  
336 turkey meat (Tagliazucchi et al. 2010; Verzelloni et al. 2010). Coffee melanoidins  
337 were the most effective respect to dark beer, barley coffee and traditional balsamic  
338 vinegar melanoidins and at a concentration of 3 mg/mL reversed the reaction and  
339 broke down hydroperoxides to a concentration lower than the initial value when  
340 digested with 300 g of turkey meat (Tagliazucchi et al. 2010). Recently, the anti-  
341 peroxidative activity of coffee melanoidins was demonstrated in an *in vivo* study  
342 (Sirota et al. 2013). The purpose of the study of Kanner and co-workers was to verify  
343 if the simultaneous consumption of 200 mL of coffee and 250 g of fast-food meat led  
344 to a reduction in the absorption of a specific advanced lipid oxidation endproducts  
345 (ALE), i.e. malondialdehyde (MDA). They measured the plasmatic level of MDA and  
346 found that the consumption of roasted coffee during a meal of fast-food meat, resulted  
347 after 2 and 4 h, in the inhibition by 80 and 50%, respectively, of post-prandial plasma  
348 MDA absorption. Although it was not possible to adequately identify the molecules  
349 (polyphenols and/or melanoidins) responsible for this effect, *in vitro* data  
350 (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 fecal microbiota resulting in the formation of acetate, propionate, and butyrate (Gniechwitz et al. 2008; 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

376 dietary antioxidants through the gastro-intestinal tract (Saura-Calixto 2011). The  
377 antioxidant bound to the dietary fibre can skip the absorption in the gut and can be  
378 released after fermentation of the carbohydrate moiety by colonic bacteria.

379 Most of these food maillardized dietary fibre carrying antioxidant compounds are  
380 poorly studied because they are not soluble in water or in the common organic  
381 solvents. Serpen et al. (2007) found that insoluble material in maillardized dietary  
382 fibre-rich foods (cereal-based foods) is able to exert a marked antioxidant activity.

383 Pérez-Jiménez et al. (2007) described a significant increase in nonextractable  
384 antioxidants associated with insoluble dietary fibre in toasted bread and bread crust as  
385 compared with wheat flour.

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

391 Food melanoidins may also act as prebiotic, able to modulate the bacterial colon  
392 population. Among the different groups present in human intestinal microbiome,  
393 *Bifidobacterium spp* and *Lactobacillus spp* are generally associated with a healthy  
394 intestinal condition, while *Clostridium spp* and *Bacterioides spp* are potentially  
395 dangerous. Bread crust melanoidins were fermented by colonic bacteria and able to  
396 selectively promote the increase in *Bifidobacterium spp* population in a static batch  
397 culture of fecal bacteria (Borrelli and Fogliano 2005). A similar effect was observed  
398 in two *in vivo* studies aimed to investigate the impact of coffee consumption on the  
399 gut bacterial population. A study carried out on human volunteers showed that the  
400 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 et al. 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; Rufián-Henares and Morales 2008b; Rufián-Henares and Morales 2009). Food melanoidins resulted 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 coffee high molecular weight 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). Barley coffee high molecular weight 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 cut-off 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 diarrhea. Rufián-Henares and Morales (2008b) tested water-soluble coffee (extracted by ultrafiltration with a cut-off of 10 kDa) melanoidins and water-insoluble biscuit (enzymatically solubilized and extracted by ultrafiltration with a cut-off 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 coffee high molecular weight 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 cocoa high molecular weight fractions (>30, 30-10, and 10-5 kDa) tested were effective in reducing the growth of *Escherichia coli* and *Enterobacter cloacea*.

## **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 United States (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

475 countries, with lower rates in middle- and high-poverty countries (Center et al. 2009).  
476 Indeed, the colorectal cancer incidence rates continue to increase in economically  
477 transitioning countries (Center et al. 2009). In part, these variations may indicate that  
478 the major causes for gastro-intestinal cancers are dietary habits and lifestyle factors  
479 (such as lack of physical activity and smoking) (Slattery et al. 1999). Excessive intake  
480 of protein, fat, and alcohol increases the risk of gastro-intestinal cancers (Willett  
481 1999). Diet is not only a risk factor for the onset of gastro-intestinal cancers but can  
482 also be preventive. Some foods, such as vegetables, beverages, and fruit have been  
483 shown to induce a chemoprotective action on the gastro-intestinal tract (Willett 1999).  
484 The most studied anti-cancer activity of food high molecular weight melanoidins  
485 involved their ability to modulate the activity of detoxifying enzymes in colon  
486 carcinoma cells model system (usually Caco-2). The detoxification from xenobiotics  
487 occurs in two phases which are called Phase I (functional group modification) and  
488 Phase II (conjugation). The most important enzymes involved in Phase I reactions are  
489 the cytochrome P450 (CYP450) isoenzymes which use oxygen and NADH, to  
490 promote the addition of a reactive hydroxyl group to the substrates. The result of this  
491 reaction is the generation of reactive molecules, which may be more reactive than the  
492 parent molecule. The Phase II detoxification reactions generally follow the Phase I  
493 reaction. Xenobiotics and carcinogen activated by the Phase I reaction, are further  
494 metabolized by Phase II conjugation reactions. The result is the conjugation of the  
495 reactive molecules with a polar group to produce more water-soluble and easy to  
496 excrete compounds. The balance between the activity of Phase I and Phase II enzymes  
497 may play a paramount role in the increased risk for different type of cancers. For  
498 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 Phase 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



524 stress during colon carcinogenesis induced by 1,2-dimethylhydrazine (Selvam et al.  
525 2009b).

526 Matrix metalloproteases (MMPs) are a class of zinc-containing endo-peptidases which  
527 are over-expressed in human colorectal cancer (Zucker and Vacirca 2004). They are  
528 involved in the degradation of extracellular matrix during the metastatic process.

529 Inhibition of MMPs synthesis and activity could be an interesting approach for colon  
530 cancer therapy together with chemotherapeutic drugs (Zucker et al. 2000). The  
531 potential inhibitory activity of coffee melanoidins against recombinant human MMPs  
532 was assayed by De Marco et al. (2011). Coffee water-soluble high molecular weight  
533 melanoidins (extracted by ultrafiltration at 10 kDa cut-off) were able to inhibit MMPs  
534 with IC<sub>50</sub> value between 0.2 and 0.7 mg/mL. Considering that the colon accumulates  
535 its content over at least 24h in a maximum volume of 2 litres, and that the daily intake  
536 of coffee melanoidins range between 0.5 and 2.0 g (Fogliano and Morales 2011), it is  
537 possible to calculate a hypothetical concentration of coffee melanoidins in the colon  
538 between 0.25 and 1 mg/mL, which are values comparable to the IC<sub>50</sub> for MMPs  
539 inhibition.

540 POTEX is a potato fibre preparation broadly used in the meat and bakery industry  
541 (Langner et al. 2011). Normally, POTEX-containing foods are thermally treated  
542 before consumption. This results in the formation of water soluble high molecular  
543 weight melanoidins from POTEX polysaccharides and proteins (Langner et al. 2011).

544 POTEX water-soluble melanoidins (isolated by ultrafiltration >10 kDa) revealed a  
545 dose-dependent antiproliferative activity against LS180 colon cancer cell line without  
546 showing any cytotoxic effect in normal colon epithelial cell line (Langner et al. 2011;  
547 Langner et al. 2013). POTEX melanoidins act through a reduction in the level of cell  
548 cycle promoters cyclin D1 and cyclin-dependent kinases and an increase in the level

549 of several cell cycle inhibitors (such as p21, p27, and p53) through ERK1/2 signalling  
550 hyper-activation.

551 Several epidemiological studies described the possible association between coffee  
552 consumption and the development of colorectal cancer. Although solid conclusions on  
553 the association between coffee consumption and risk of colon cancer has not been  
554 obtained yet, some recent meta-analysis of prospective cohort studies seem to suggest  
555 the existence of an inverse relationship between coffee consumption and colorectal  
556 cancer risk. In a meta-analysis of 12 prospective cohort studies, Je and co-workers  
557 (2009) concluded that coffee drinkers do not have a decreased risk of colorectal, colon  
558 or rectal cancer. Interestingly, they found a marginally lower incidence of colon  
559 cancer in women who drank more than 4 cups of coffee per day. In a subsequent  
560 meta-analysis carried out on 15 prospective cohort studies, Yu et al. (2011) suggested  
561 that coffee consumption has an inverse association with some type of cancers  
562 including colon cancer. In a very recent meta-analysis of 16 prospective cohort  
563 studies, Li and colleagues (2013) found a slight inverse association between coffee  
564 consumption and colorectal and colon cancer.

565 Given this consideration, it is surprising that literature is lacking in investigations  
566 focused on the direct effects of coffee bioactive compounds (including melanoidins)  
567 on colon cancer. Recently, Vitaglione et al. (2012) reviewed the possible mechanisms  
568 by which coffee bioactives (chlorogenic acids and melanoidins) may influence the  
569 risk of colorectal cancer development. Three possible pathways correlating coffee  
570 intake to the reduction of colorectal cancer risk were suggested as follows: (1)  
571 increase in colon motility which result in an increased carcinogen elimination rate  
572 (coffee dietary fibre and melanoidins); (2) modulation of gut microbiota which could  
573 result in an amelioration of insulin sensitivity and body weight loss, reducing colon

574 cancer risk (coffee dietary fibre and melanoidins); and (3) reduction in the  
575 inflammation in colon mucosa by coffee antioxidants resulting in a reduced colon  
576 cancer risk (melanoidins). Although the hypothesis are speculative and not  
577 investigated till now, their conclusions should be considered the starting point to  
578 study the possible ability of coffee melanoidins/dietary fibre to positively influence  
579 the colon function.

580 Very recently, Argirova and colleagues (2013), demonstrated *ex vivo* the ability of  
581 coffee water-soluble melanoidins (isolated by ultrafiltration, cut-off 5 kDa) to induce  
582 contractions in gastric smooth muscle. Coffee melanoidins provoked a depolarization  
583 of smooth muscle membranes which resulted in an increased influx of  $\text{Ca}^{2+}$  into the  
584 cell. Coffee melanoidins were able to induce the contraction of gastric smooth muscle  
585 cells by interacting with muscarinic acetylcholine receptors.

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

623 different biological properties and physiological activities. For this reason an  
624 important future effort must be made to isolate and purify the various structures  
625 within a food.

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

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

640

**Conflict of interest**

The authors declare that they have no conflict of interest.

## References

- Acar O, Gokmen V, Pellegrini N et al (2009) Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. *Eur J Food Sci Technol* 229:961–969
- Alamir I, Niquet-Leridon C, Jacolot P et al (2013) Digestibility of extruded proteins and metabolic transit of N-epsilon-carboxymethyllysine in rats. *Amino Acids* 44:1441–1449
- Argirova, MD, Stefanova ID, Krustev AD (2013) New biological properties of coffee melanoidins. *Food Funct* 4:1204–1208
- Bekedam EK, Loots MJ, Schols HA et al (2008) Roasting effects on formation mechanisms of coffee brew melanoidins. *J Agric Food Chem* 56:7138–7145
- Bellesia A, Tagliazucchi D (2014) Cocoa brew inhibits in vitro  $\alpha$ -glucosidase activity: The role of polyphenols and high molecular compounds. *Food Res Int* 63:439–445
- Borrelli RC, Visconti A, Mennella C et al (2002) Chemical characterization and antioxidant properties of coffee melanoidins. *J Agric Food Chem* 50:6527–6533
- Borrelli RC, Mennella C, Barba F et al (2003) Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food Chem Toxicol* 41:1367–1374
- Borrelli RC, Fogliano V (2005) Bread crust melanoidins as potential prebiotic ingredients. *Mol Nutr Food Res* 49:673–678
- Center MM, Jemal A, Ward E (2009) International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev* 18:1688–1694
- Coelho C, Ribeiro M, Cruz ACS, Dominques MRM, Coimbra MA, Bunzel M, Nunes FM (2014) Nature of phenolic compounds in coffee melanoidins. *J Agric Food Chem* 62:7843–7853

- Crowe FL, Key TJ, Appleby TN et al (2012) Dietary fibre intake and ischaemic hearth disease mortality: the European Prospective Investigation into Cancer and Nutrition-Hearth study. *Eu J Clin Nutr* 66 :950–956
- Daglia M, Tarsi R, Papetti A et al (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
- De Marco LM, Fischer S, Henle T (2011) High molecular weight coffee melanoidins are inhibitors for matrix metalloproteases. *J Agric Food Chem* 59:11417–11423
- Del Pino-García R, González-SanJosé ML, Rivero-Pérez MD et al (2012) Influence of degree of roasting on the antioxidant capacity and genoprotective effect of instant coffee: contribution of the melanoidin fraction. *J Agric Food Chem* 60:10530–10539
- Delgado-Andrade C, Tessier FJ, Niquet-Leridon C et al (2013) Study of the urinary and faecal excretion of N (epsilon)-carboxymethyllysine in young human volunteers. *Amino Acids* 43:595–602
- Delgado-Andrade C (2014) Maillard reaction products: some considerations on their health effects. *Clin Chem Lab Med* 52:53–60
- Edwards BK, Ward E, Kohler BA et al (2010) Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* 116:544–573
- Faist V, Erbersdobler HF (2001) Metabolic transit and *in vivo* effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Ann Nutr Met* 45:1–12



- Finot PA, Magnenat E (1981) Metabolic transit of early and advanced Maillard products. *Prog Food Nutr Sci* 5:193–207
- Fogliano V, Morales FJ (2011) Estimation of dietary intake of melanoidins from coffee and bread. *Food Funct* 2:117–123
- Förster A, Kühne Y, Henle T (2005) Studies on absorption and elimination of dietary maillard reaction products. *An N Y Acad Sci* 1043:474–481
- Gniechwitz D, Reichardt N, Meiss E et al (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 et al (2008b) Isolation and characterization of a coffee melanoidin fraction. *J Sci Food Agric* 88:2153–2160
- Goya L, Delgado-Andrade C, Rufián-Henares JA et al (2007) Effect of coffee melanoidins on human hepatoma HepG2 cells. Protection against oxidative stress induced by *tert*-butylhydroperoxide. *Mol Nutr Food Res* 51:536–545
- Hellwig M, Matthes R, Peto A, Löbner J, Henle T (2014) N- $\epsilon$ -fructosyllysine and N- $\epsilon$ -carboxymethyllysine, but not lysinoalanine, are available for the absorption after simulated gastrointestinal digestion. *Amino Acids* 46:289–299
- Hiramoto S, Itoh K, Shizuuchi S et al (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 model systems. *J Agric Food Chem* 1:928–943
- Icatlo FC, Kuroki M, Kobayashi C et al (2003) Affinity purification of *Helicobacter pylori* urease: relevance to gastric mucosa adherence by urease protein. *J Biol Chem* 273:18130–18138

- Jaquet M, Rochat I, Moulin J et al (2009) Impact of coffee consumption on the gut microbiota: a human volunteer study. *Int J Food Microbiol* 130:117–121
- Je Y, Liu W, Giovannucci E (2009) Coffee consumption and risk of colorectal cancer: a systematic review and meta-analysis of prospective cohort studies. *Int J Cancer* 124:1662–1668
- Kuntcheva MJ, Obretenov TD (1996) Isolation and characterization of melanoidins from beer. *Z Lebensm Unters Forsch* 202:238–243
- Lamb A, Chen LF (2013) Role of the *Helicobacter pylori*-induced inflammatory response in the development of gastric cancer. *J Cell Biochem* 114:491–497
- Langner E, Nunes FM, Pożarowski P et al (2011) Antiproliferative activity of melanoidins isolated from heated potato fiber (Potex) in glioma cell culture model. *J Agric Food Chem* 59:2708–2716
- Langner E, Nunes FM, Pożarowski P et al (2013) Melanoidins isolated from heated potato fiber (Potex) affect human colon cancer cells growth via modulation of cell cycle and proliferation regulatory proteins. *Food Chem Toxicol* 57:246–255
- Leclercq C, Arcella D, Piccinelli R et al (2009) The Italian National Food Consumption Survey INRAN-SCAI 2005–06: main results in terms of food consumption. *Public Health Nutr* 12:2504–2532
- Li W, Khor TO, Xu C et al (2008) Activation of Nrf2-antioxidant signaling attenuates NF- $\kappa$ B-inflammatory response and elicits apoptosis. *Biochem Pharmacol* 76:1485–1489
- Li G, Ma D, Zhang Y et al (2013) Coffee consumption and risk of colorectal cancer: a meta-analysis of observational studies. *Public Health Nutr* 16:346–357
- 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
- Martin MA, Ramos S, Mateos R et al (2009) Biscuit melanoidins of different molecular masses protect human HepG2 cells against oxidative stress. *J Agric Food Chem* 57:7250–7258
- Mesías M, Seiquer I, Navarro MP (2009a) Influence of diets rich in Maillard reaction products on calcium bioavailability. Assays in male adolescents and in Caco-2 cells. *J Agric Food Chem* 57:9532–9538
- Mesías M, Seiquer I, Delgado-Andrade C et al (2009b) Intake of Maillard reaction products reduces iron bioavailability in male adolescents. *Mol Nutr Food Res* 53:1551–1560
- Morales FJ, Somoza V, Fogliano V (2012) Physiological relevance of dietary melanoidins. *Amino Acids* 42:1097–1109
- Moreira ASP, Fernando MN, Domingues R et al (2012) Coffee melanoidins: structures, mechanisms of formation and potential health impacts. *Food Funct* 3:903–915
- Murphy N, Norat T, Ferrari P et al (2012) Dietary fibre intake and risks of cancers of the colon and rectum in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLoS One* 7:e39361
- Nakano M, Kubota M, Owada S et al (2013) The pentosidine concentration in human blood specimens is affected by heating. *Amino Acids* 44:1451–1456
- Nakayama T, Oishi K (2013) Influence of coffee (*Coffea arabica*) and galacto-oligosaccharide consumption on intestinal microbiota and the host response. *FEMS Microbiol Lett* 343:161–168

- 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
- Papetti A, Pruzzo C, Daglia M et al (2007) Effect of barley coffee on the adhesive properties of oral streptococci. *J Agric Food Chem* 55:278–284
- Pastoriza S, Rufián-Henares JA (2014) Contribution of melanoidins to the antioxidant capacity of the Spanish diet. *Food Chem* 164:438–445
- Pérez-Jiménez J, Díaz-Rubio ME, Mesías M, Morales FJ, Saura-Calixto F (2014) Evidence for the formation of maillardized insoluble dietary fiber in bread: A specific kind of dietary fiber in thermally processed food. *Food Res Int* 55:391–396
- Reichardt N, Gniechwitz D, Steinhart H et al (2009) Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota. *Mol Nutr Food Res* 53:287–299
- Rivero D, Pérez-Magariño S, González-Sanjose ML et al (2005) Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and melanoidins. *J Agric Food Chem* 53:3637–3642
- Rombouts I, Lagrain B, Brijs K et al (2012) Cross-linking of wheat gluten proteins during production of hard pretzels. *Amino Acids* 42:2429–2438
- Ronca G, Palmieri L, Maltinti S et al (2003) Relationship between iron and protein content of dish and polyphenol content in accompanying wines. *Drugs Exp Clin Res* 29:271–286
- Roncero-Ramos I, Delgado-Andrade C, Alonso-Olalla R et al (2012) Effects of bread crust-derived Maillard reaction products on phosphorous balance in rats. *Eur J Nutr* 51:871–879

- Roncero-Ramos I, Delgado-Andrade C, Haro A et al (2013a) Effects of dietary bread crust Maillard reaction products on calcium and bone metabolism in rats. *Amino Acids* 44:1409–1418
- Roncero-Ramos I, Delgado-Andrade C, Morales FJ et al (2013b) Influence of Maillard products from bread crust on magnesium bioavailability in rats. *J Sci Food Agric* 93:2002–2007
- Roncero-Ramos I, Delgado-Andrade C, Tessier FJ et al (2013c) Metabolic transit of N(ε)-carboxymethyl-lysine after consumption of AGEs from bread crust. *Food Funct* 4:1032–1039
- Roncero-Ramos I, Niquet-Léridon C, Strauch C et al (2014) An advanced glycation end product (AGE)-rich diets promoted N(ε)-carboxymethyl-lysine accumulation in the cardiac tissue and tendons of rats. *J Agric Food Chem* 62:6001–6006
- 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) Microtiter plate-based assay for screening antimicrobial activity of melanoidins against *E. coli* and *S. aureus*. *Food Chem* 111:1069–1074
- Rufián-Henares JA, Morales FJ (2008b) Antimicrobial activity of melanoidins against *Escherichia coli* is mediated by a membrane-damage mechanism. *J Agric Food Chem* 56:2357–2362
- Sauer T, Raithel M, Kressel J et al (2013) Activation of the transcription factor Nrf2 in macrophages, Caco-2 cells and intact human gut tissue by Maillard reaction products and coffee. *Amino Acids* 44:1427–1439

- Saura-Calixto F (2011) Dietary fiber as a carrier of dietary antioxidants; an essential physiological function. *J Agric Food Chem* 59:43–49
- Selvam JP, Aranganathan S, Nalini N (2009a) Inhibitory effect of bread crust antioxidant pronyl lysine on two different categories of colonic premalignant lesions induced by 1,2-dimethylhydrazine. *Eu J Cancer Prev* 18:291–302
- Selvam JP, Aranganathan S, Gopalan R et al (2009b) Chemopreventive efficacy of pronyl-lysine on lipid peroxidation and antioxidant status in rat colon carcinogenesis. *Fundam Clin Pharmacol* 23:293–302
- Senadheera D, Cvitkovitch DG (2008) Quorum sensing and biofilm formation by *Streptococcus mutans*. *Adv Exp Med Biol* 631:178–188
- Serpen A, Capuano E, Fogliano V, Gökmen V (2007) A new procedure to measure the antioxidant activity of insoluble food components. *J Agric Food Chem* 55:7676–7681
- Silvan JM, Morales FJ, Saura-Calixto F (2010) Conceptual study on maillardized dietary fiber in coffee. *J Agric Food Chem* 58:12244–12249
- Sirota R, Gorelik S, Harris RM et al (2013) Coffee polyphenols protect human plasma from postprandial carbonyl modifications. *Mol Nutr Food Res* 57:916–909
- Slattery ML, Edwards SL, Boucher KM et al (1999) Lifestyle and colon cancer: an assessment of factors associated with risk. *Am J Epidemiol* 150:869–877
- Somoza V, Wenzel E, Lindenmeier M et al (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 et al (2006) Dose-dependent utilization of casein-linked lysinoalanine, *N*(epsilon)-fructoselysine and *N*(epsilon)-carboxymethyllysine in rats. *Mol Nutr Food Res* 50:833–841

- Stauder M, Papetti A, Mascherpa D et al (2010) Antiadhesion and antibiofilm activities of high molecular weight coffee components against *Streptococcus mutans*. J Agric Food Chem 58:11662–11666
- Summa C, McCourt J, Cämmerer B et al (2008) Radical scavenging activity, anti-bacterial 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 (2008) Antioxidant properties of traditional balsamic vinegar and boiled must model systems. Eur Food Res Tech 227 :835–843
- Tagliazucchi D, Verzelloni E, Conte A (2010) Effect of dietary melanoidins on lipid peroxidation during simulated gastro-intestinal digestion: their possible role in the prevention of oxidative damage. J Agric Food Chem 58:2513–2519
- Tagliazucchi D, Verzelloni E (2014) Relationship between the chemical composition and the biological activities of food melanoidins. Food Sci Biotechnol 23:561–568
- Verzelloni E, Tagliazucchi D, Conte A (2010) From balsamic to healthy: traditional balsamic vinegar melanoidins inhibit lipid peroxidation during simulated gastric digestion of meat. Food Chem Toxicol 48:2097–2102
- Verzelloni E, Tagliazucchi D, Del Rio D et al (2011) Antiglycative and antioxidative properties of coffee fractions. Food Chem 124:1430–1435
- Vitaglione P, Fogliano V, Pellegrini N (2012) Coffee, colon function, and colorectal cancer. Food Funct 3:916–912
- Wang H, Qian H, Yao W (2011) Melanoidins produced by the Maillard reaction: Structure and biological activity. Food Chem 128:573–584
- Wilkinson J, Clapper ML (1997) Detoxification enzymes and chemoprevention. Proc Soc Exp Biol Med 216:192–200

Willett WC (1999) Goals for nutrition in the year 2000. *CA Cancer J Clin* 49:331–352

Yu X, Bao Z, Zou J et al (2011) Coffee consumption and risk of cancers: a meta-analysis of cohort studies. *BMC Cancer* 11:96

Zucker S, Cao J, Chen WT (2000) Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* 56:6642–6650

Zucker S, Vacirca J (2004) Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev* 23:101–117



**Table 1.** Structures and components of food melanoidins.

<i>Product</i>	<i>Structures</i>	<i>Components</i>	<i>Ref</i>
Coffee	Carbohydrate-based	Galactomannans, arabinogalactan 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. 2010a; Tagliazucchi and Verzelloni 2014
Dark Beer	Carbohydrate-based	Glucose, proteins, phenolic moieties, Maillard reaction products	Rivero et al. 2005; Tagliazucchi et al. 2010a; 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

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

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

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

Biological activity	Biological effect	Food melanoidins	Ref.
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. 2010a; Del Pino et al. 2012; Vitaglione et al. 2012; Sauer et al. 2013
Chelating ability	Reduction in carcinogen formation ( <i>N</i> -nitroso compound) and reduction in cytotoxicity	Coffee, barley coffee, dark beer, and traditional balsamic vinegar melanoidins	Tagliazucchi et al. 2010a; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014