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

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1 **Abstract**

2

3 Emerging evidence from laboratory researches have highlighted the bioactivity of  
4 food melanoidins and melanoproteins. Whilst such studies have been carried out with  
5 different *in vitro* systems, information about melanoidins absorption and bio-

6 availability are scarce. However, they are generally considered as poorly absorbable  
7 and bio-available compounds. Therefore, we present a review in which the gastro-  
8 intestinal tract is hypothesized to be the main site of action of food melanoidins and  
9 melanoproteins biological activity. We described recent data supporting this

10 hypothesis both *in vitro* model systems and *in vivo*. Importantly, we focused this  
11 review only on the effect of melanoidins and melanoproteins extracted from food.

12 Most of the studies had been carried out using water-soluble carbohydrate-based  
13 melanoidins isolated from different food sources (beer, barley coffee, coffee). In

14 bakery products, melanoidins are protein-based structure (melanoproteins) which are  
15 largely insoluble in water. Dietary melanoidins and melanoproteins have been

16 demonstrated to exert *in vitro* antioxidant and metal chelating ability in the gastro-  
17 intestinal tract reducing the formation of lipid hydroperoxides and advanced lipid

18 oxidation end-products during the digestion of meat. The reduction in the formation of  
19 these pro-atherogenic compounds has been shown to be followed by a decrease in

20 their absorption in human volunteers. Food melanoidins have also shown *in vitro* anti-  
21 caries and prebiotic activities. We conclude, underlining the possible role of food

22 melanoidins in the prevention of gastro-intestinal tract cancers. We hope this review  
23 will stimulate further research on food melanoidins and their biological activities in

24 the gastro-intestinal tract.

25

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

28

## 29 **Introduction**

30

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

43 Melanoidins are generically defined as brown-coloured, nitrogen-containing, high  
44 molecular weight compounds (Hodge 1953). Their chemical structure is still largely  
45 unknown despite their presence in a large range of thermally treated food products  
46 such as coffee, bread, biscuits, meat, barley coffee, beer, cocoa, and traditional  
47 balsamic vinegar (Summa et al. 2008; Tagliacruzchi et al. 2008; Tagliacruzchi et al.  
48 2010; Fogliano and Morales 2011; Moreira et al. 2012).

49 Considering the high intake of melanoidins (Fogliano and Morales 2011), their  
50 biological activity and potential impact on human health is a topic of great interest.  
51 Different *in vitro* biological activities have been attributed to melanoidins, namely,  
52 antioxidant, antimicrobial, prebiotic, anti-cancer, antihypertensive and anti-glycative

53 activities (Rufián-Henares and Morales 2007; Rufián-Henares and Morales 2008a  
54 2009; Verzelloni et al. 2011; Borrelli and Fogliano 2005; Vitaglione et al. 2012).  
55 Two major factors limit the actual physiological relevance of the biological activities  
56 of melanoidins. First, the limited knowledge of the structure of food melanoidins  
57 makes it difficult to identify the active principles responsible for the specific  
58 biological activity. Most studies have been carried out using the high molecular  
59 weight material (usually higher than 10 kDa) isolated from foods and beverages  
60 without further purification. Secondly, although melanoidins are consumed regularly  
61 as part of the daily human diet, they are generally considered as poorly absorbable and  
62 poorly bio-available compounds (Faist and Erbersdobler 2001).

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

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

77

78 **Structural and chemical characteristics of food melanoidins and melanoproteins**

79

80 The elucidation of the chemical and structural properties of melanoidins and  
81 melanoproteins is an important research area in food science and even though many  
82 efforts have been waged in the last years, the structural properties of food melanoidins  
83 are still largely unknown. The prominent difficulty in the study of the structure of  
84 food melanoidins is a consequence of their diversity and heterogeneity, that reflect the  
85 complexity of the starting substrates, i.e. foods. Foods and beverages in fact contain  
86 numerous possible reagents which may be involved in the formation of melanoidins,  
87 such as amino acids, peptides, proteins, simple sugars and complex carbohydrates,  
88 polyphenols, etc.. Therefore, distinct melanoidin populations, with different chemical  
89 (e.g. molecular weight, charge) and structural (depending on the nature of reactants)  
90 properties can be present in food (**Table 1**). Very recent review papers and research  
91 articles focused on this topic (Fogliano and Morales 2011; Wang et al. 2011; Moreira  
92 et al. 2012; Tagliazucchi and Verzelloni 2014; Pastoriza and Rufián-Henares 2014).  
93 In some foods such as coffee, cocoa, traditional balsamic vinegar, sweet wine and  
94 barley-derived beverages, most of the melanoidins are carbohydrate-based structures  
95 whereas in other foods (bakery foods) they are protein-based structures  
96 (melanoproteins). In addition to proteins/amino acids and carbohydrates, also other  
97 compounds can be incorporated into food melanoidins during their formation (**Table**  
98 **1**).

99

100 **Estimation of melanoidins and melanoproteins content in food and their dietary**  
101 **intake**

102

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

128 amount of melanoproteins in the bread crusts ranged from 14 to 30 g per 100 g of  
129 crust, depending on the type of bread but it decreased to 4.4 g per 100 g in the whole  
130 bread (Fogliano and Morales 2011; Pastoriza and Rufián-Henares 2014). Furthermore  
131 the amount of melanoproteins found in dry biscuits ranged between 12 and 20 g per  
132 100 g of whole product, whereas in breakfast cereals it was higher (25.5 g per 100 g).  
133 For the calculation of the daily intake the authors referred to a study published by the  
134 Italian National Institute of Nutrition (INRAN) (Leclercg et al. 2009) which reported  
135 an average bread consumption among the Italian population of 103.3 g per day with a  
136 mean consumption among Italian bread consumers of 112.1 g per day. The same  
137 statistical research was made regarding the consumption of biscuits, defining an  
138 average intake of 13.8 g in Italian population with mean consumption of 27.3 g per  
139 day in consumers. Regarding breakfast cereals the average consumption was  
140 estimated at 1.5 and 14.1 g per day in Italian population and consumers, respectively.  
141 Combining the consumption data with the content of melanoproteins in bread, biscuits  
142 and breakfast cereals, the dietary intake of melanoproteins for bakery products can be  
143 estimated at around 6.5 g per day for average population and 12.3 g per day for  
144 consumers, respectively.

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

149 There are different factors such as the temperature and time of fermentation process,  
150 type of grain used and colour which affect the melanoidin content of beer. Dark beer  
151 made using roasted malt or roasted barley showed a melanoidins content between 0.15  
152 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.

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

181

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

184

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

189 Firstly, after the consumption of foods and beverages rich in melanoidins, such  
190 compounds can be present in the stomach and intestinal lumen at high concentrations,  
191 compatible with those shown *in vitro* biological effects. Secondly, although  
192 melanoidins are consumed regularly as part of the daily human diet, they are generally  
193 considered as poorly absorbable and poorly bio-available compounds (Faist and  
194 Erbersdobler 2001). The absorption of the melanoidins depends on their molecular  
195 weight and solubility (Finot and Magneat 1981; Alamir et al. 2013; Nakano et al.  
196 2013; Delgado-Andrade et al. 2013; Hellwig et al. 2014). The absorption of the low  
197 molecular weight and water soluble melanoidins seems to be favoured. In rats 70 to  
198 90% of orally ingested high molecular weight melanoidins (> 10 kDa and prepared  
199 from amino acid/glucose and casein/glucose model systems) are excreted in faeces,  
200 and only 1 to 5% absorbed and excreted in urine. Interestingly, the metabolic transit  
201 was similar for the melanoidins from both model systems (Finot and Magneat 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 Magneat 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.

251

252 **Antioxidant properties of food melanoidins in the gastro-intestinal tract**

253

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

260 Rufián-Henares and Morales (2007) evaluated the impact of simulated gastro-  
261 pancreatic digestion on the radical scavenger ability of water-soluble coffee  
262 melanoidins isolated by ultrafiltration with a nominal cut-off of 10 kDa using several  
263 cell-free assays. They found that coffee melanoidins retained their radical scavenger  
264 ability even after the passage in the *in vitro* digestion system. Coffee high molecular  
265 weight melanoidins, therefore, seem not to be degraded in the first portion of the  
266 gastro-intestinal tract. A recent paper by Del Pino-García et al. (2012) showed that  
267 water-soluble high molecular weight melanoidins (> 10 kDa) extracted from coffee  
268 and submitted to *in vitro* gastro-intestinal digestion exhibited high radical scavenger  
269 activity assayed with FRAP, ABTS, and DPPH methods. Also, the cold-water soluble  
270 high molecular weight fractions of coffee brews isolated by ultrafiltration and  
271 subjected to *in vitro* fermentation for 24h with human faecal bacteria still showed  
272 antioxidant properties (Reichardt et al. 2009).

273 Recently, a series of papers published by our group (Tagliacruzchi et al. 2010;  
274 Verzelloni et al. 2010; Tagliacruzchi and Verzelloni 2014) showed that water-soluble  
275 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

301 biscuit melanoproteins resulted in a protective effect against the oxidative stress  
302 induced by *t*-BOOH, albeit less effective than the coffee melanoidins.  
303 Antioxidant properties of food melanoidins can result from their free radical  
304 scavenging activity but their ability to chelate transition metal ions also plays an  
305 important role. Dietary melanoidins are able to bind Ca<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, and  
306 Fe<sup>2+</sup> (Morales et al. 2012). The chelating ability of food melanoidins arises from their  
307 anionic nature which is strongly pH-dependent. Melanoidins exert a net negative  
308 electric charge at pH 5.0 and become more negative at higher pH values (Morales et  
309 al. 2012). High molecular weight water-soluble melanoidins (> 10 kDa) extracted  
310 from different foods maintained the ability to chelate iron under gastric conditions  
311 (Tagliacruzchi et al. 2010; Verzelloni et al. 2010; Tagliacruzchi and Verzelloni 2014).  
312 Coffee melanoidins were more effective in chelate free iron ions respect to traditional  
313 balsamic vinegar, barley coffee and dark beer melanoidins (Tagliacruzchi and  
314 Verzelloni 2014). Binding of ions in the gastro-intestinal tract may have negative  
315 health effects, possibly reducing the absorption and bio-availability of these ions.  
316 Mesías et al. (2009a) examined the effect of a diet rich in MRP on calcium bio-  
317 availability in healthy male adolescents. No significant changes in calcium bio-  
318 availability were observed between the MRP-rich and the MRP-poor diet. The same  
319 group tested on rats the effect of bread crust MRP on calcium, magnesium and  
320 phosphate bio-availability (Roncero-Ramos et al. 2012; Roncero-Ramos et al. 2013a;  
321 Roncero-Ramos et al. 2013b). They concluded that the bio-availability of the tested  
322 ions was unmodified by consumption of bread crust or its isolate fractions. On the  
323 contrary, the bio-availability of iron was reduced by 2.7 fold in male adolescents who  
324 consumed a MRP-rich diet respect to the group fed with a MRP-poor diet (Mesías et  
325 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

351 melanoidins are mainly responsible for the anti-peroxidative effect of coffee found *in*  
352 *vivo*.

353

#### 354 **Food melanoidins as dietary fibre and prebiotic**

355

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

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

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

374 Indeed, almost all of the chemically characterized food maillardized soluble and  
375 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

401 population of *Bifidobacterium spp* (Jaquet et al. 2009). A more recent *in vivo* study  
402 was carried out on mice fed for 3 days with coffee (Nakayama et al. 2013). After  
403 coffee consumption, *Escherichia coli* and *Clostridium spp* counts significantly  
404 decreased in the proximal colon whereas the *Bifidobacterium spp* population  
405 increased in the same area.

406

#### 407 **Antimicrobial and anti-caries activity of food melanoidins**

408

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

417 Regarding the possible relevance for the gastro-intestinal tract, particular emphasis  
418 should be given to the anti-cariogenic potential of food melanoidins. The most  
419 important pathogenic bacteria involved in the development of dental caries is the  
420 Gram-positive bacteria *Streptococcus mutans*. Its cariogenic potential is in part related  
421 to its ability to adhere to the tooth surface and form a bio-film (Senadheera and  
422 Cvitkovitch 2008). In a first study, Daglia et al. (2002) reported the anti-adhesive  
423 effect of green and roasted coffee. Both coffees tested were able to inhibit the  
424 adsorption of *S. mutans* to saliva coated hydroxyapatite. More interesting, roasted  
425 coffee samples were significantly more active than the corresponding green coffee

426 samples. In a subsequent work by the same group, water-soluble coffee melanoidins  
427 were unequivocally identified as *in vitro* anti-cariogenic compounds in roasted coffee  
428 (Stauder et al. 2010). The whole high molecular weight fraction of roasted coffee (>  
429 3.5 kDa) at concentration of 6 mg/mL showed potent adhesion inhibitory activity  
430 (91% of inhibition), antimicrobial activity and inhibitory activity against *S. mutans*  
431 bio-film formation (100% of inhibition). The coffee high molecular weight fraction  
432 was subsequently fractionated using gel filtration chromatography. The obtained  
433 melanoidin fractions were active against *S. mutans* adhesion and bio-film formation.  
434 Barley coffee melanoidins have been also tested for their anti-cariogenic activity *in*  
435 *vitro* (Papetti et al. 2007). Barley coffee high molecular weight fraction (> 1 kDa and  
436 consisting of water-soluble melanoidins) displayed anti-adhesive and anti-bio-film  
437 properties. The high molecular weight fraction of barley coffee was further  
438 fractionated using a combination of dialysis and gel filtration chromatography. The  
439 most active fraction was found to consist of a single brown component with molecular  
440 weight higher than 1000 kDa.

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

450 A variety of high molecular weight food melanoidins were also able to exert  
451 antimicrobial activity against *Escherichia coli*, a Gram-negative bacteria which is  
452 non-desirable in a large presence in the gut microflora and can cause severe diarrhea.  
453 Rufián-Henares and Morales (2008b) tested water-soluble coffee (extracted by  
454 ultrafiltration with a cut-off of 10 kDa) melanoidins and water-insoluble biscuit  
455 (enzymatically solubilized and extracted by ultrafiltration with a cut-off of 10 kDa)  
456 melanoproteins for their antimicrobial activity against *E. coli*. The antimicrobial  
457 activity was expressed as MIC (minimum inhibitory concentration), defined as the  
458 lowest concentration of melanoidin fractions not producing any detected cell growth  
459 (Rufián-Henares and Morales 2008b). Biscuit melanoproteins demonstrated higher  
460 antimicrobial activity (MIC value 7.5 mg/mL) than coffee high molecular weight  
461 melanoidins (MIC value 10 mg/mL). In another study (Rufián-Henares and Morales  
462 2008a), the same authors showed that coffee melanoidins had higher antimicrobial  
463 activity than beer melanoidins. Summa et al. (2008) reported that all the cocoa high  
464 molecular weight fractions (>30, 30-10, and 10-5 kDa) tested were effective in  
465 reducing the growth of *Escherichia coli* and *Enterobacter cloacea*.

466

#### 467 **The possible role of food melanoidins in the protection of gastro-intestinal tract** 468 **cancers**

469

470 Gastro-intestinal tract tumours are one of the most common forms of neo-plastic  
471 diseases affecting humans. In particular colon-rectal cancer represents the second  
472 most frequent cause of cancer death in the United States (Edwards et al. 2010). The  
473 incidence of gastro-intestinal cancers varies greatly depending on the geographical  
474 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-

499 transferase (GST), have been identified and associated with increased risk for colon  
500 cancer (Wilkinson and Clapper 1997).

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

513 *In vivo* effects of malt, bread crust, and pronylated protein were tested in a 15-day  
514 animal trial on rats (Somoza et al. 2005). As a result, feeding of 5% bread crust  
515 resulted in a 18% elevated activity of GST in the kidneys whereas the administration  
516 of pronyl bovine serum albumin (BSA) caused an increase of 27% of liver UDP-  
517 glucuronyl transferase. In two additional *in vivo* studies, the chemopreventive  
518 potential of pronyl-lysine extracted from bread crust was assayed using rats treated  
519 with the carcinogen 1,2-dimethyl hydrazine. Pronyl-lysine was able to reduce the total  
520 aberrant crypt foci formation, total number of dysplastic foci, and cell proliferation in  
521 the colon, suggesting that pronyl-lysine suppresses 1,2-dimethylhydrazine-induced  
522 colon carcinogenesis effectively (Selvam et al. 2009a). The anti-cancer effect of  
523 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 afflux of Ca<sup>2+</sup> 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).

598 An additional mechanism which could be related to the anti-cancer activity of  
599 melanoidins in the gastro-intestinal tract is their heme-binding ability. Heme can act  
600 as a catalyst for oxidative damage and can initiate colorectal cancer (Tagliacruzchi et  
601 al. 2010; Verzelloni et al. 2010; Tagliacruzchi and Verzelloni 2014). Dietary water-  
602 soluble melanoidins were able to bind heme under gastro-intestinal conditions  
603 (Tagliacruzchi et al. 2010; Verzelloni et al. 2010; Tagliacruzchi and Verzelloni 2014).  
604 Coffee melanoidins had greater affinity towards heme in comparison to barley coffee,  
605 dark beer, and traditional balsamic vinegar melanoidins (Tagliacruzchi and Verzelloni  
606 2014). Melanoidins may act in the gastro-intestinal tract as "sponges" capable of  
607 sequestering the heme groups released during the digestion of meat and delivering  
608 them to the faeces where they are then excreted.

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

611

## 612 **Conclusion**

613 In recent years an increasing number of studies have been published regarding the  
614 possible effects of melanoidins in the gastro-intestinal tract. Due to their low  
615 bioavailability, it is unlikely that melanoidins can exert their protective effects at the  
616 systemic level. More plausibly, melanoidins can act at gastro-intestinal level where  
617 they reach high concentration following dietary intake. Most of the studies have been  
618 carried out *in vitro* and suffer some limitations concerning mainly the lack of  
619 knowledge about the structure of melanoidins. It is becoming increasingly clear that in  
620 foods a single type of melanoidin does not exist but different melanoidin populations  
621 co-exist within a single sample. Indeed, the results obtained until now have  
622 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, Tagliacruzchi 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, Dominiques 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- $\epsilon$ -fructosylllysine 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 f N( $\epsilon$ )-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( $\epsilon$ )-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
- Tagliacruzchi D, Verzelloni E, Conte A (2008) Antioxidant properties of traditional balsamic vinegar and boiled must model systems. *Eur Food Res Tech* 227 :835–843
- Tagliacruzchi 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
- Tagliacruzchi D, Verzelloni E (2014) Relationship between the chemical composition and the biological activities of food melanoidins. *Food Sci Biotechnol* 23:561–568
- Verzelloni E, Tagliacruzchi 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, Tagliacruzchi 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; Tagliacruzchi 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	Tagliacruzchi et al. 2010a; Tagliacruzchi and Verzelloni 2014
Dark Beer	Carbohydrate-based	Glucose, proteins, phenolic moieties, Maillard reaction products	Rivero et al. 2005; Tagliacruzchi et al. 2010a; Tagliacruzchi and Verzelloni 2014
Cocoa	Carbohydrate-based	Polysaccharides, proteins, polyphenols (catechins)	Summa et al. 2008; Bellesia and Tagliacruzchi 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