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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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25/04/2024 08:55

(Article begins on next page)

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 Ca²⁺, Pb²⁺, Co²⁺, Zn²⁺, Cu²⁺, and
306 Fe²⁺ (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 IC₅₀ 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 IC₅₀ 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 Ca²⁺ 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 (Tagliazucchi et
601 al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014). Dietary water-
602 soluble melanoidins were able to bind heme under gastro-intestinal conditions
603 (Tagliazucchi et al. 2010; Verzelloni et al. 2010; Tagliazucchi and Verzelloni 2014).
604 Coffee melanoidins had greater affinity towards heme in comparison to barley coffee,
605 dark beer, and traditional balsamic vinegar melanoidins (Tagliazucchi 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.

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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