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Effects of iron, ascorbate, meat and casein on the antioxidant capacity of green tea under conditions of in vitro digestion

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Abstract

The hypothesis that interactions of dietary polyphenols with dietary iron occur during digestion and result in a decrease of the post-absorptive antioxidant properties of polyphenols was investigated. The hypothesis was tested in vitro, under conditions that simulate gastrointestinal digestion. Mixtures of green tea, iron, and three dietary factors that modify the form of iron in the lumen, namely ascorbic acid, meat or casein, were subjected to an in vitro gastrointestinal digestion. Antioxidant capacity (FRAP assay), iron concentration (ferrozine assay) and polyphenol concentration (Folin–Ciocalteau assay) were measured in the in vitro digests. The presence of iron decreased the antioxidant capacity and the polyphenol concentration of green tea digests. The presence of ascorbic acid increased, while meat and casein decreased the antioxidant capacity of green tea. The factorial analysis of the data suggests that protein and iron interact with green tea polyphenols during the in vitro digestion and decrease their antioxidant capacity. These results support the aforementioned hypothesis.

Keywords: Antioxidant; Green tea; Iron; In vitro; Polyphenols

1. Introduction

There is limited information concerning the antioxidant capacity of polyphenols in the presence of dietary iron or other dietary factors. Our hypothesis is that dietary iron interacts with polyphenols in the lumen during digestion and, consequently, decreases the antioxidant capacity of polyphenols.

The antioxidant capacity of polyphenols and of food sources of polyphenols has been repeatedly shown in various in vitro and in vivo systems in the absence of dietary factors (Frankel, Kanner, German, Parks, & Kinsella, 1993; Khan, Katiyar, Agarwal, & Mukhtar, 1992; Lionis, Faresjo, Skoula, Kapsokefalou, & Faresjo, 1998; Matsingou, Kapsokefalou, & Salifoglou, 2000, 2001, 2003). Epidemiological evidence suggests that the antioxidant properties of polyphenols may impart health benefits (Hollman & Katan, 1999; Riemersma, Rice-Evans, Tyrrell, Clifford, & Lean, 2001; Serafini, Bellocco, Wolk, & Ekstrom, 2002).

Despite the growing interest in the health benefits of polyphenols, little is known about whether dietary factors, that may interact with polyphenols during digestion, affect the antioxidant capacity of polyphenols in plasma. One dietary factor that interacts with polyphenols is protein (Zhu, Phillipson, Greengrass, Bowery, & Cai, 1997). Limited experimental evidence suggests that milk diminishes the antioxidant properties of tea (Arts et al., 2002) and dark chocolate (Serafini et al., 2003). Another dietary factor that most likely interacts with polyphenols is iron. Lumenal interactions of polyphenols with iron are frequently addressed in studies of iron absorption. Iron absorption is inhibited by polyphenols and dietary sources of polyphenols, such as tea, possibly through formation of unavailable iron–polyphenol chelates in the lumen during digestion (Hallberg & Hulthen, 2000; Samman et al., 2001; Zijp, Korver, &

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Tijburg, 2000). The formation of iron–polyphenol chelates is not easily predictable in more complicated systems that contain dietary factors that modify the form of iron (Hallberg & Hulthen, 2000), such as meat or ascorbic acid or casein or other proteins (Kapsokefalou & Miller, 1991).

The objective of this study was to test, in vitro, the hypothesis that lumenal interactions of dietary polyphenols with dietary iron modulate the antioxidant properties of polyphenols. Mixtures of iron, green tea and ascorbic acid, meat or casein were subjected to a simulated gastrointestinal digestion. Subsequently, the antioxidant capacity of the digests was measured in vitro. The effects of protein and iron valence on the antioxidant behaviour of polyphenols in vitro were investigated.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich (Steinheim, Germany). Doubly-distilled, deionized water was used throughout the experiment. All glassware was washed, soaked overnight in 1 N HCl and rinsed with distilled deionised water.

The materials used in the in vitro digestion experiment were the following: pepsin was a porcine pepsin preparation (4 g), suspended in 100 ml 0.1 M HCl. Pancreatin/bile mixture was a porcine pancreatin (0.2 g) and a crude bile extract (1.2 g) suspended in 100 ml of 0.1 M $NaHCO_3$. PIPES buffer, 0.15 M PIPES (piperazine-*N*,*N*′-bis[2-ethane-sulfonic acid] disodium salt), was adjusted to pH 6.3 using concentrated HCl. HEPES buffer, 0.3 M HEPES (*N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid sodium salt) was used without pH adjustment. Protein precipitant solution (reducing) was 100 g trichloroacetic acid, 50 g hydroxylamine monohydrochloride and 100 ml concentrated HCl per 1 l of water. Protein precipitant solution (non-reducing) was prepared as was the reducing solution, except that the hydroxylamine monohydrochloride was not added. Ferrozine chromogen solution (5 mg/ml) was ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4 triazine, disodium salt) in water. Spectrapore® I dialysis tubing, with a molecular weight cut-off of 6000–8000 (Spectrum Laboratories, Rancho Dominguez, CA, USA), was cut into 25 cm lengths and soaked in water for at least 1 h prior to use and stored in 0.15 M PIPES pH 6.3, until used.

2.2. Sample preparation

Infusions of green tea (Java Green Tea, R. Twining & Co, Ltd.) were prepared by steeping 4 g of tea/100 ml boiling water for 3 min and subsequently filtering through filter paper. Meat was lean beef, bought from a local supermarket. The meat was minced, shaped into patties, about $2 \times 2 \times 1$ cm and of 30 g each, and frozen at −20 °C until further use. On the day of the experiment, the patties to be used for testing were thawed and cooked in a microwave oven (at high) for 2 min one side and 1 min the other side.

Samples of 20 ml were prepared by mixing iron with green tea and with ascorbic acid (tea + AA), casein (tea + casein) or meat (tea + meat) at the following concentrations: the sample concentration of iron was 0.25 or 1 mM. The iron source was a standard solution of $FeCl_3$, 1010 ppm in 1 M HCl, from Sigma–Aldrich, Germany. Samples containing meat or casein provided 4 g of protein/100 ml sample. Samples containing ascorbic acid provided 88 mg ascorbic acid/100 ml sample. Each sample was homogenised and digested in vitro as described below.

2.3. In vitro digestion

The in vitro model employed herein simulates the gastrointestinal digestion by subjecting samples to a 4.5 h incubation at 37 °C, at different pHs, in the presence of pepsin, pancreatin and bile extract and by fractionating digests through a dialysis membrane (Kapsokefalou & Miller, 1991). Briefly, samples of 20 ml, pH adjusted to 2.8 with concentrated HCl, were transferred into 120 ml screw-cap vials and placed in a shaking water bath maintained at 37 °C. The samples were incubated for 2 h in the presence of 1 ml pepsin suspension, added to each sample. At the end of this incubation, the pH of the samples was adjusted gradually from 2.8 to around 6 with the aid of a dialysis sac, filled with 20 ml of a 0.15 M PIPES buffer, pH 6.3. The dialysis sac was immersed in the incubating samples. After 30 min, 5 ml of a pancreatin–bile salt mixture (0.5 and 3 g, respectively, in 250 ml 0.1 M $NaHCO_3$) were added to the samples and the incubation continued for another 2 h. At the end of this incubation period, the dialysis sac was removed. The dialysate (fraction inside the dialysis bag) and the retentate (fraction outside the dialysis bag) were collected. The retentate was centrifuged at 10,000*g* for 10 min. The supernatant was removed and kept for further analysis.

2.4. Analysis

The antioxidant capacity was estimated from the reducing power of the samples (FRAP assay) according to Benzie and Strain (1996). Results were expressed as increase in absorbance 4 min after the addition of the FRAP reagent. Ferrous and total (ferrous + ferric) iron were measured using a modification of the ferro-

zine method proposed by Reddy, Chidambaram, Fonseca, and Bates (1986) as in Kapsokefalou and Miller (1991).

Total polyphenol content was determined in all samples and in the in vitro digests according to a modification of the Folin–Ciocalteu method (Kähkönen et al., 1999). The total phenolic content was expressed in milligrammes of gallic acid equivalents per ml digest (mg GAE/ml).

2.5. Data analysis

Samples were run in duplicate and each experiment was repeated three times. Differences among samples were tested with one-way ANOVA, LSD test at 95% confidence interval, after testing for normality (Zar, 1999). Data were also analysed according to a 4×3 factorial design, for factors dietary factor and iron, and according to a 2×3 factorial design, for factors protein and iron. Analysis of data was carried out with the programme Statistica, version 5.1 (StatSoft, OK, USA).

3. Results and discussion

The antioxidant capacity and the concentration of polyphenols and of soluble ferrous and total iron were measured on the dialysates and on the supernatant of the centrifuged retentates (Figs. 1–4). These two fractions consist of different compounds; therefore results obtained have different implications. The dialysates consist of compounds that dialyse through the porous membrane, namely soluble compounds of molecular weight smaller than 6000. The retentates consist of soluble and insoluble compounds of various molecular weights; however, soluble compounds, with molecular weights above 6000, may be found only in the supernatant of the centrifuged retentatess.

The antioxidant capacity of the dialysates is presented in Fig. 1, graph A. In the absence of iron, the antioxidant capacities of the dialysates of the tea + AA digests was higher than that of the dialysates of tea digests ($P < 0.05$). Addition of casein or meat to tea before digestion, decreased the antioxidant capacity of the dialysates of the digests ($P < 0.05$). There was no difference between the effect of meat and of casein on the antioxidant capacity of tea digests ($P > 0.05$).

Most interestingly, the addition of iron at 0.25 mM and at 1 mM before the in vitro digestion in tea, tea + AA, tea + casein or tea + meat, decreased the antioxidant capacities of the dialysates of all digests. This reduction was statistically significant in all treatments when iron was added at 1 mM ($P < 0.05$). However, at the lower iron level, a trend towards decrease of the antioxidant capacity was also observed. It must be emphasized that the iron concentrations of 0.25 mM and of 1 mM are the iron concentrations of the mixtures before digestion, corresponding to the iron concentration in a meal before ingestion, i.e., to 3.5 and 14 mg of iron per 250 ml of tea. These amounts compare well with the suggested daily intake of iron.

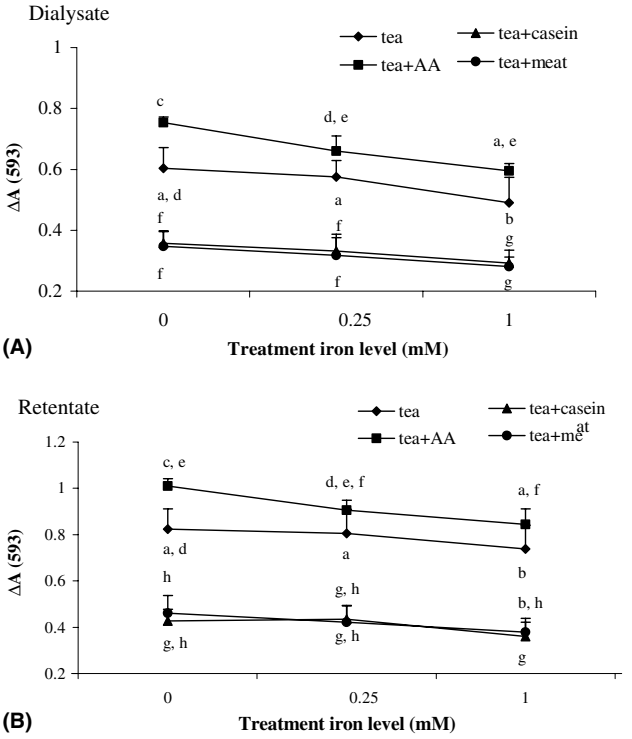

Fig. 1. Antioxidant capacity of tea digested in vitro in the presence of iron and of ascorbic acid, casein or meat. The antioxidant capacity was measured in the dialysate and the retentate of the in vitro digests (graph A and graph B, respectively) by the FRAP assay and was expressed as change in absorbance monitored at 593 nm, 4 min after the addition of the chromagen mixture. Means with different letters are significantly different ($P < 0.05$).

Differences of the antioxidant capacities of the dialysates of the various digested treatments observed in the absence of iron were also observed in the presence of iron at both concentration levels. In particular, the antioxidant capacity of the dialysates of tea + AA digests was higher than that of the dialysates of the tea digests ($P < 0.05$) and that of dialysates of tea + casein and tea + meat digests was lower at both concentration levels of iron ($P < 0.05$).

The factorial statistical analysis shows that iron or protein, added before digestion to the various tea treatments, modulates the antioxidant capacity of the dialysates of tea digests ($P < 0.05$). However, there is no interaction between iron and protein for the expression of this effect ($P > 0.05$).

The comparisons among the retentates of the digests of the various treatments and the results of the statistical analysis were similar to those reported for the respective dialysates (Fig. 1, graph B).

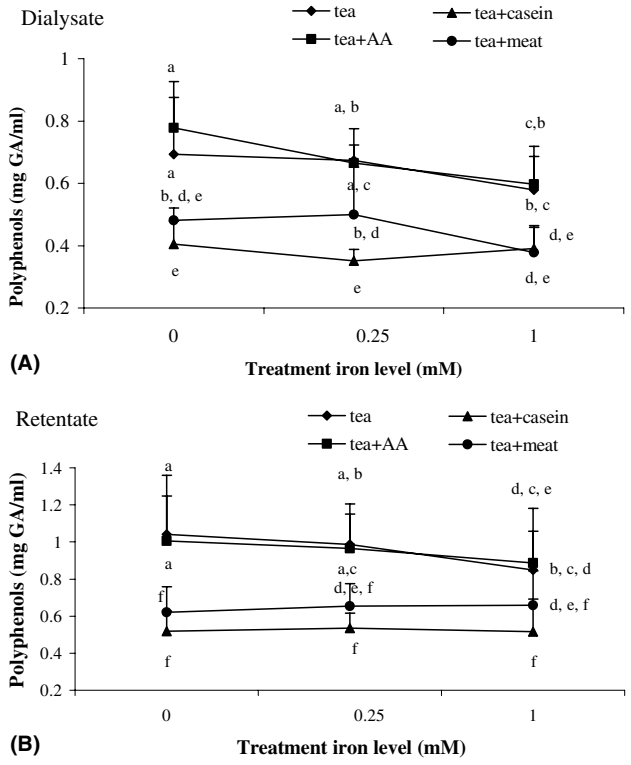

Fig. 2. Polyphenol content of tea digested in vitro in the presence of iron and of ascorbic acid, casein or meat. Polyphenol content was determined in the dialysate and the retentate of the in vitro digests (graph A and graph B, respectively) with the Folin–Ciocalteau assay and was expressed as milligrammes of gallic acid equivalents per ml digest (mg GAE/ml). Means with different letters are significantly different ($P < 0.05$).

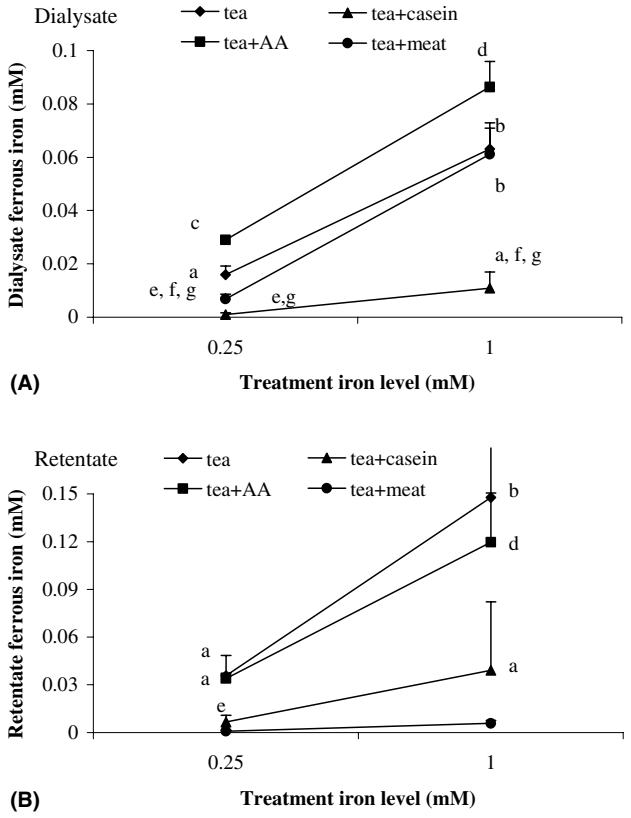

Fig. 3. Ferrous iron content of tea digested in vitro in the presence of iron and of ascorbic acid, casein or meat. Iron was measured in the dialysate and the retentate of the in vitro digests (graph A and graph B, respectively) by the ferrozine assay. Means with different letters are significantly different ($P < 0.05$).

The polyphenol content of the dialysates is presented in Fig. 2, graph A. In the absence of iron, the polyphenol contents of the dialysates of tea and tea + AA digests were similar ($P > 0.05$). The addition of casein or meat to tea before digestion decreased the polyphenol content of the dialysates of the tea digests ($P < 0.05$). There was no difference between the effect of meat and of casein on the polyphenol content of the dialysates of the tea digests ($P > 0.05$). The addition of iron at 0.25 mM and at 1 mM, before the in vitro digestion to tea, tea + AA, tea + casein or in tea + meat, decreased the polyphenol content in the dialysates of the digests. This reduction was statistically significant ($P < 0.05$) only when iron was added at 1 mM to tea and tea + AA digests. There was no change in polyphenol content in the dialysates of the digests in tea + casein or in tea + meat ($P > 0.05$).

Differences in the polyphenol content of the dialysates of the various treatments digested in vitro were observed in the presence of iron at both levels. In particular, the polyphenol contents of the dialysates of the tea and of tea + AA digests were higher than those of dialysates of tea + casein and tea + meat digests at both levels of iron.

The factorial statistical analysis shows that iron, added before digestion to the various tea treatments, modulates the polyphenol content and the antioxidant capacity of the dialysates of tea digests. Moreover, it shows that protein, also added before digestion to the various tea treatments, modulates the antioxidant capacity and the polyphenol content of the dialysates of tea digests ($P < 0.05$). However, iron and protein do not interact statistically ($P > 0.05$).

The polyphenol content of the retentates is presented in Fig. 2 graph B. The comparisons among the retentates of the digests of the various treatments and the results of the statistical analysis were similar to those reported for the respective dialysates.

The soluble iron content was measured in the dialysate and the retentate of the in vitro digests of all treatments (Figs. 3 and 4). Most of the soluble iron measured was in the ferrous form. Ascorbic acid increased ferrous and total iron formation in both the dialysate and the retentate. In contrast to observations on the antioxidant capacity and polyphenol formation, meat and casein affected iron formation in a different manner. In particular, casein decreased ferrous and total iron formation in both the dialysate and the retentate, while meat did not.

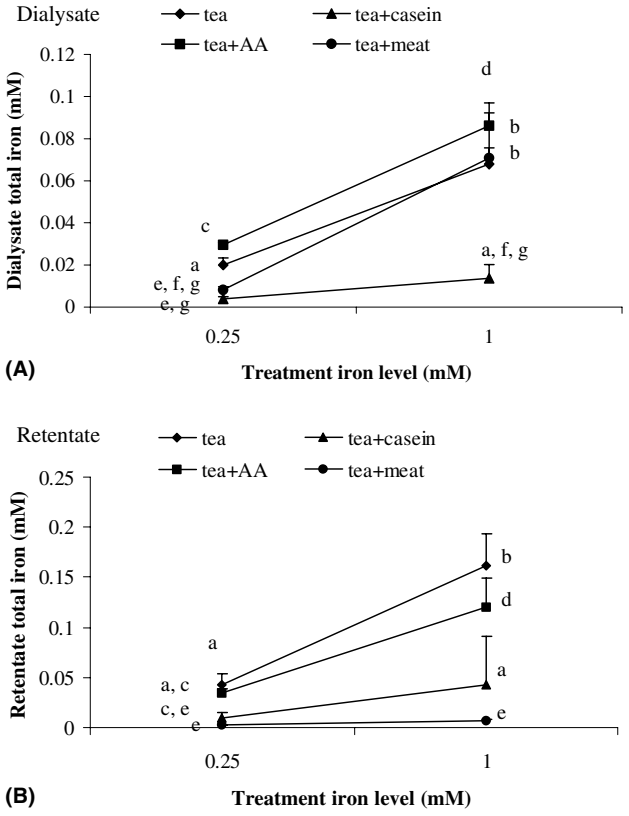

Fig. 4. Total iron content of tea digested in vitro in the presence of iron and of ascorbic acid, casein or meat. Iron was measured in the dialysate and the retentate of the in vitro digests (graph A and graph B, respectively) by the ferrozine assay. Means with different letters are significantly different ($P < 0.05$).

The first finding emerging from the present study was that iron may be an important modulator of the antioxidant capacity of tea. The observed decrease of the antioxidant capacity of tea when iron is present depends on the amount of iron added to tea before digestion but not in the form of iron. Variation in the form of iron in the in vitro digests was induced by ascorbic acid, casein and meat. These dietary components altered the physicochemical form of iron under lumenal conditions. Meat and ascorbate reduce iron and form soluble, low molecular weight chelates of ferrous iron, while casein forms high molecular weight chelates of ferric iron under simulated gastrointestinal conditions (Kapsokefalou & Miller, 1991). The presence of these selected dietary factors in the in vitro digests was expected to greatly affect the valence and/or the ligands iron is chelated to and, consequently, the iron–polyphenol interaction. The expected effect of the dietary factors on the formation of total and ferrous iron in the digests was observed (Figs. 3 and 4); however, the factorial analysis showed that there was no effect of interaction of iron and dietary factors on the decrease of the antioxidant activity.

The second finding was that iron may decrease the concentration of polyphenols of tea digests in the lumen. This finding provides evidence for the iron–polyphenol interaction. Therefore, it is plausible that the observed decrease in antioxidant capacity may be attributed to physicochemical interactions of polyphenols with iron in the lumen. These interactions result in the formation of iron–polyphenol chelates that may exert low antioxidant capacity. The formation of iron–polyphenol chelates has been observed in model systems and involves iron in the ferric state and adjacent phenolic oxygens of the polyphenols (Jovanovic, Simic, Steenken, & Hara, 1998; Kawabata, Schepkin, Haramaki, Phadke, & Packer, 1996). The formation of iron–polyphenol chelates in the lumen may be responsible for the inhibition of iron absorption when tea, or other food sources of polyphenols, is present in an iron containing meal (Hurrell, Reddy, & Cook, 1999). Herein we report, for the first time, that, in our in vitro system, iron–polyphenol interactions may result in a decrease in antioxidant capacity of tea. It has been suggested that one mechanism whereby polyphenols exert their antioxidant effect in vivo is through chelation of oxidation catalysts, such as iron (Yoshino & Murakami, 1998). This chelation has been proposed to occur in the plasma and to involve absorbed polyphenol and iron species. Attention to the lumenal antioxidant effect of polyphenols is directed toward non-absorbed polyphenol species (Halliwell, Zhao, & Whiteman, 2000). Lumenal interactions that may affect post absorptive antioxidant events have not been explored and are the subject of the hypothesis tested herein in vitro.

Our third finding was that protein, either from meat or from casein, decreases the antioxidant capacity of green tea digests. Protein was expected to interact, not only with iron, but also with polyphenols. Most interestingly, however, the decrease in antioxidant capacity of tea digests is similar when meat or casein is present, despite the reducing power and antioxidant capacity that meat and casein have (Kitts & Weiler, 2003). This suggests that the effect of protein is very strong and prevails over other antioxidant characteristics of meat. In some studies, it has been observed that milk, a good source of casein, decreases the antioxidant capacity of tea (Arts et al., 2002; Serafini, Ghiselli, & Ferro-Luzzi, 1996). However, other studies have not observed an effect of milk (Hollman, Van Het Hof, Tijburg, & Katan, 2001; Leenen, Roodenburg, Tijburg, & Wiseman, 2000). Recently it was shown that milk may also reduce the antioxidant capacity of chocolate (Schroeter, Holt, Orozco, Schmitz, & Keen, 2003; Serafini et al., 2003). No observation of the effect of meat on the antioxidant capacity of tea has been reported.

Central to the in vitro study of iron–polyphenol–protein interactions is the in vitro gastrointestinal digestion to which all samples were subjected. The in vitro digestion simulates the gastrointestinal environment and processes, integrates chemical transformations of the

dietary components during the digestive procedure, such as hydrolysis, polymerisation or oxidation, allows chemical interactions between the digestive components to occur and provides separation of the soluble low MW fraction. This latter feature is of significance because it may be related to the fraction that is potentially available for absorption. Although this simulated digestion process has been developed and tested for iron bioavailability studies, it offers advantages that are relevant to the study of polyphenols over other model systems. Besides allowing interactions between polyphenols and other dietary components to occur under digestive conditions, other important advantages are the pH changes to which polyphenols are sensitive (Friedman & Jurgens, 2000) and the separation of fractions based on molecular weight. The molecular weight is presumably a significant factor that determines polyphenol absorption (Scalbert & Williamson, 2000). For that reason, antioxidant measurements, performed in the soluble, low molecular weight fraction of the digests, are evaluated carefully. Therefore, the procedure employed herein is an excellent tool for the in vitro evaluation of various factors that may affect iron–polyphenol interactions proposed to occur during the course of digestion. Nevertheless, in vitro results on the antioxidant properties of polyphenols present limitations and may not necessarily reflect in vivo effects. Other physiological factors, such as absorption and metabolism of polyphenols, are important in evaluating the activity of polyphenols in vivo (Rice-Evans, 2001).

4. Conclusions

Under in vitro conditions that simulate the gastrointestinal digestion, the antioxidant capacity of green tea decreases when iron is present before digestion.

Iron may be an important modulator of the antioxidant capacity of tea. In particular, this experiment shows that presence of iron decreased the antioxidant capacity and the polyphenol content of tea digests under conditions that simulate the gastrointestinal digestion. These findings support the hypothesis that interactions between iron and polyphenols occur in the lumen and result in a decrease of the antioxidant capacity of polyphenols.

References

Arts, M. J., Haenen, G. R., Wilms, L. C., Beetstra, S. A., Heijnen, C. G., Voss, H. P., et al. (2002). Interactions between flavonoids and proteins: effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry, 50*(5), 1184–1187.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry, 239*(1), 70–76.

Frankel, E. N., Kanner, J., German, J. B., Parks, E., & Kinsella, J. E. (1993). Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *Lancet, 341*(8843), 454–457.

Friedman, M., & Jurgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry, 48*(6), 2101–2110.

Hallberg, L., & Hulthen, L. (2000). Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition, 71*(5), 1147–1160.

Halliwell, B., Zhao, K., & Whiteman, M. (2000). The gastrointestinal tract: a major site of antioxidant action? *Free Radical Research, 33*(6), 819–830.

Hollman, P. C. J., & Katan, M. B. (1999). Health effects and bioavailability of dietary flavonols. *Free Radical Research, 31*, S75–S80.

Hollman, P. C., Van Het Hof, K. H., Tijburg, L. B., & Katan, M. B. (2001). Addition of milk does not affect the absorption of flavonols from tea in man. *Free Radical Research, 34*(3), 297–300.

Hurrell, R. F., Reddy, M., & Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition, 81*(4), 289–295.

Jovanovic, S. V., Simic, M. G., Steenken, S., & Hara, Y. (1998). Iron complexes of gallocatechins. Antioxidant action or iron regulation?. *Journal of the Chemical Society, Perkin Trans, 2*, 2365–2369.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*(10), 3954–3962.

Kapsokefalou, M., & Miller, D. D. (1991). Effects of meat and selected food components on the valence of nonheme iron during in vitro digestion. *Journal of Food Science, 56*(2), 352–355, 358.

Kawabata, T., Schepkin, V., Haramaki, N., Phadke, R. S., & Packer, L. (1996). Iron coordination by catechol derivative antioxidants. *Biochemical Pharmacology, 51*(11), 1569–1577.

Khan, S., Katiyar, S. K., Agarwal, R., & Mukhtar, H. (1992). Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 Hairless mice: possible role in cancer chemoprevention. *Cancer Research, 52*(14), 4050–4052.

Kitts, D. D., & Weiler, K. (2003). Bioactive proteins and peptides from food sources. Application of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design, 9*(16), 1309–1323.

Leenen, R., Roodenburg, A. J., Tijburg, L. B., & Wiseman, S. A. (2000). A single dose of tea with or without milk increases plasma antioxidant activity in humans. *European Journal of Clinical Nutrition, 54*(1), 87–92.

Lionis, C., Faresjo, A., Skoula, M., Kapsokefalou, M., & Faresjo, T. (1998). Antioxidant effects of herbs in Crete. *The Lancet, 352*(9145), 1987–1988.

Matsingou, T. C., Kapsokefalou, M., & Salifoglou, A. (2000). In vitro antioxidant activity of black tea and mediterranean herb infusions toward iron under simulated gastrointestinal conditions. *Journal of Food Science, 65*, 1060–1065.

Matsingou, T. C., Kapsokefalou, M., & Salifoglou, A. (2001). Aqueous infusions of Mediterranean herbs exhibit antioxidant activity toward iron promoted oxidation of phospholipids, linoleic acid and deoxyribose. *Free Radical Research, 35*(5), 593–605.

Matsingou, T. C., Petrakis, N., Kapsokefalou, M., & Salifoglou, A. (2003). Antioxidant activity of organic extracts from aqueous infusions of sage. *Journal of Agricultural and Food Chemistry, 51*(23), 6696–6701.

Rice-Evans, C. (2001). Flavonoid antioxidants. *Current Medicinal Chemistry, 8*(7), 797–807.

Riemersma, R. A., Rice-Evans, C. A., Tyrrell, R. M., Clifford, M. N., & Lean, M. E. (2001). Tea flavonoids and cardiovascular health. *QJM: Monthly Journal of the Association of Physicians, 94*(5), 277–282.

Reddy, M. B., Chidambaram, M. V., Foneca, J., & Bates, G. W. (1986). Potential role of in vitro iron bioavailability studies in combating iron deficiency: a study of the effects of phosvitin on iron mobilization from pinto beans. *Clinical Physiology and Biochemistry, 4*(1), 78–86.

Samman, S., Sandstrom, B., Toft, M. B., Bukhave, K., Jensen, M., Sorensen, S. S., & Hansen, M. (2001). Green tea or rosemary extract added to foods reduces nonheme-iron absorption. *American Journal of Clinical Nutrition, 73*(3), 607–612.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, 130*(8S), 2073S–2085S.

Schroeter, H., Holt, R. R., Orozco, T. J., Schmitz, H. H., & Keen, C. L. (2003). Milk and absorption of dietary flavanols. *Nature, 426*(6968), 787–788.

Serafini, M., Bellocco, R., Wolk, A., & Ekstrom, A. M. (2002). Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology, 123*(4), 985–991.

Serafini, M., Bugianesi, R., Maiani, G., Valtuena, S., De Santis, S., & Crozier, A. (2003). Plasma antioxidants from chocolate. *Nature, 424*(6952), 1013.

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A. (1996). In vivo antioxidant effect of green and black tea in man. *European Journal of Clinical Nutririon, 50*(1), 28–32.

Yoshino, M., & Murakami, K. (1998). Interaction of iron with polyphenolic compounds: Application to antioxidant characterization. *Analytical Biochemistry, 257*, 40–44.

Zar, J. H. (1999). Multiple comparisons. In *Biostatistical analysis* (pp. 208–230). Englewood Cliffs, NJ: Prentice Hall.

Zhu, M., Phillipson, J. D., Greengrass, P. M., Bowery, N. E., & Cai, Y. (1997). Plant polyphenols: biologically active compounds or non-selective binders to protein. *Phytochemistry, 44*(3), 441–447.

Zijp, I. M., Korver, O., & Tijburg, L. B. (2000). Effect of tea and other dietary factors on iron absorption. *Critical reviews in food science and nutrition, 40*(5), 371–398.