Food Chemistry 112 (2009) 46–50

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Impact of phytochemical-rich foods on bioaccessibility of mercury from fish

Soon-Mi Shim [a], Mario G. Ferruzzi [b], Young-Cheul Kim [c], Elsa M. Janle [a], Charles R. Santerre [a,*]

[a] Department of Foods and Nutrition, Purdue University, Stone Hall, Room 205, 700 W. State Street, West Lafayette, IN 47907-2059, United States
[b] Department of Food Science, Purdue University, 745 Agricultural Mall Dr. West Lafayette, IN 47907-2009, United States
[c] Department of Nutrition, University of Massachusetts, Amherst, MA 01003-9282, United States

ARTICLE INFO

Article history:
Received 26 December 2007
Received in revised form 2 April 2008
Accepted 9 May 2008

Keywords:
Mercury
Bioaccessibility
Phytochemical
In vitro digestion

ABSTRACT

The effects of phytochemical-rich foods on bioaccessibility of mercury in fish tissue (the amount of mercury that is released from fish into gastrointestinal tract fluid following a simulated digestion) were investigated using an *in vitro* digestion. Total mercury in the aqueous phase following a simulated digestion of fish with added food treatments was used to measure mercury bioaccessibility. Green tea extract (31–2000 mg), black tea extract (31–2000 mg), and soy protein (50–100 mg) significantly reduced mercury bioaccessibility by 82–92%, 88–91%, and 44–87%, respectively. Grapefruit juice (0.5–10 ml) did not reduce mercury in the aqueous phase. Wheat bran (50–1000 mg) decreased mercury bioaccessibility (84%); oat bran and psyllium reduced bioaccessibility (by 59–75%, 15–31%, respectively) at amounts greater than 500 mg. We therefore suggest that co-consumption of foods containing phytochemicals at the same time as fish that contains mercury may potentially reduce mercury absorption compared to eating fish alone.

1. Introduction

Fish is a source of high-quality protein, vitamin D, selenium, omega-3 fatty acids, and other nutrients. Recently, omega-3 fatty acids, including eicosapentaenoic acid (EPA, C22:5*n*−3) and docosahexaenoic acid (DHA, C22:6*n*−3), were found to play a role in brain and retina development and function (Allen & Harris, 2001; Lauritzen, Hansen, Jorgensen, Michaelsen et al., 2001). Fish, however, has also been identified as the main source of methylmercury exposure in humans (Marsh, Clarkson, Myers et al., 1995; NAS, 2000). In general, more than 90% of the mercury in fish is found as methylmercury (US EPA (US Environmental Protection Agency), 1997). Following absorption, methylmercury accumulates in brain, muscle, and kidney, presumably due to its strong affinity for sulfhydryl groups (US EPA, 1997; US FDA, 2004). Additionally, methylmercury can cross the placental–blood and blood–brain barriers (Marsh et al., 1995). Excessive prenatal exposure can result in developmental delays (Rogers, Emmett, Ness, Golding, et al., 2004; US EPA, 1997). As a result, the US Environmental Protection Agency (US EPA) has established a reference dose (RfD) for methylmercury of 0.1 μg Hg/kg body weight per day (US EPA (US Environmental Protection Agency), 1997).

Therapeutic treatment following exposure to mercury often involves administration of chelating agents, such as 2, 3-dimercapto-1-propane sulfonate (DMPS) (Aposhian et al., 1992; NAS, 2000). Chelating agents are believed to reduce the bioaccessibility of methylmercury, thereby limiting the ability of the heavy metal to be absorbed or reabsorbed in the intestine. Some studies have suggested that particular dietary factors, including fibres and phytochemicals, can similarly impact methylmercury bioavailability (Chapman & Chan, 2000; Minamisawa, Minamisawa, Yoshida, Takai et al., 2004; Ou, Kongrong & Li, 1999; Rowland, Mallett, Flynn, Hargreaves et al., 1986). For instance, co-consumption of wheat bran with methylmercury simultaneously increased fecal excretion of mercury and decreased methylmercury levels in blood and brain in animal studies (Rowland et al., 1986). Thiol-containing compounds in garlic are believed to act as metal-chelating or complexing agents which can increase mercury excretion (Cha, 1987; NAS, 2000; Rhee, Cha, & Bae, 1985). It has also been suggested that biologically active components in tea and coffee may inhibit the adsorption of heavy metals such as cadmium and lead (Minamisawa et al., 2004). Phytochemicals are compounds in plant-derived foods that are commonly found in the diet, and believed to provide health benefits which may include preventing chronic disease (Cha, 1987; NAS, 2000). However, information about the specific effects of these food components on human intestinal absorption of methylmercury is lacking.

Bioaccessibility is defined as the amount of a chemical that is released from food into gastrointestinal tract fluid following a simulated digestion and, as a result, is available for absorption by the intestinal mucosa (Garret, Failla, & Sarama, 1999; Oomen et al., 2003; Versantvoort, Oomen, Kamp, Rompelberg, Sips et al., 2005). By virtue of high throughput and lower cost, this *in vitro* technique

* Corresponding author. Tel.: +1 765 496 3443; fax: +1 765 494 0674.
E-mail address: santerre@purdue.edu (C.R. Santerre).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.030

has been used to assess bioaccessibility of nutrients, phytochemicals and contaminants (Ferruzzi, Failla, & Schwartz, 2001, 2002; Garret et al., 1999; Glahn, Lee, Yeung, Goldman, Miller et al., 1998; Liu, Glahn, & Liu, 2004). For instance, bioaccessibility has been used to assess bioavailability, including cadmium from vegetables, aflatoxin B_1 from peanuts and ochratoxin A from buckwheat (Oomen et al., 2003; Versantvoort, Kamp, & Rompelberg, 2004). A simulated gastrointestinal digestion offers a rapid and less expensive approach for estimating the bioaccessibility of toxins and toxicants (Versantvoort et al., 2005).

The primary goal of this study was to determine the effects of foods that are rich in phytochemicals and dietary fibre (e.g., green tea, black tea, soy protein, grapefruit juice, wheat bran, oat bran and psyllium) on mercury bioaccessibility, using an *in vitro* digestion model. Therefore our main hypotheses are the following: (1) phytochemicals (e.g., catechin and isoflavone)/dietary fibres (insoluble fibre) will bind mercury in the gut, making it insoluble and unavailable for absorption by intestinal mucosa; (2) phytochemical-containing foods will bind mercury in a dose-dependent manner.

2. Materials and methods

2.1. Materials

King mackerel was obtained from the Florida Department of Environmental Protection (Tallahassee, FL) and analysis found it to contain 1 ppm of total mercury. 2, 3-Dimercapto-1-propane sulfonic acid (DMPS, reagent grade) and sodium copper chlorophyllin (SCC, commercial grade) were purchased from Sigma-Aldrich (St. Louis, MO). Green and black tea powdered extracts were kindly provided by Plantextrakt (Vestenbergsgreuth, Germany) and its characterization is described in Table 1. Isolated soy protein (Table 2), used for beverage, was obtained from Protein Technologies International Checker Board (Square St., Louis, MO). Ruby red grapefruit juice (100% pure squeezed grapefruit juice, Tropicana Product Inc. Chicago, IL) was purchased from a local market (Payless, Kroger Co., West Lafayette, IN). Both oat bran (CFO) and hard red wheat bran (Lot No. 195) were purchased from the American Association of Cereal Chemists (AACC, St. Paul, MN). Psyllium (Psyllium Hydrophilic Mucilloid, Product No. 3178) was provided by Proctor & Gamble Co. (Mason, OH).

2.2. Test meals preparation

Fish tissue was homogenized in a food processor with stainless-steel blades (HC 3000, Proctor-Silex, Inc., Washington, NC). A 5 g portion of fish homogenized tissue (5 μg of mercury) and 5 ml of saline (0.9% NaCl, Sigma–Aldrich) were twice homogenized using a cell disruptor (Cell disruptor 185, Branson Sonic Power Co., Danbury, CT) at 20 kHz at 150–500 Watts for 30 s. To produce a finished test meal, homogenized fish tissue was mixed with one of the following: green tea powder (31, 62.5, 125, 250, 500, 1000 and 2000 mg), black tea powder (31, 62.5, 125, 250, 500, 1000, and 2000 mg), soy protein powder (50, 100, 250, 500 and 1000 mg), grapefruit juice (0.5, 2, 5 and 10 ml), oat bran (50, 100, 500 and 1000 mg), red hard wheat bran (50, 100, 500 and 1000 mg), psyllium (50, 100, 500 and 1000 mg), or DMPS (1.25, 2.5, 5, 10, 20 and 50 μg).

Table 1
Specification of the green tea/black tea powdered extract

Ingredient	Green tea[a]	Black tea[b]
Catechins (HPLC) based on dry matter	30%	NA[c]
Caffeine (HPLC) based on dry matter	5–10%	3%

[a] Data from Plantextrakt, Vestenbergsgreuth, Germany.
[b] Green tea powdered extract is a spray-dried extract, made from green tea. Black tea powdered extract is a brown spray-dried extract made from tea.
[c] Not available.

Table 2
Isoflavone (IF) composition of soy protein isolates

Phytochemical	μg/g product
Daidzin	264
Glycitin	34.8
Genistin	383
Malonyl daidzin	320
Malonyl glycitin	33.2
Malonyl genistin	640
Acetyl daidzin	121
Acetyl glycitin	0.00
Acetyl genistin	158
Daidzein	0.00
Glycitein	0.00
Genistein	248
Total	2198

[a] Data from Dr. Connie Weaver's Laboratory Foods and Nutrition Department, Purdue University, West Lafayette, IN, USA (For beverage, 29 g of soy protein/8–10 fl oz of water, milk, or beverage).

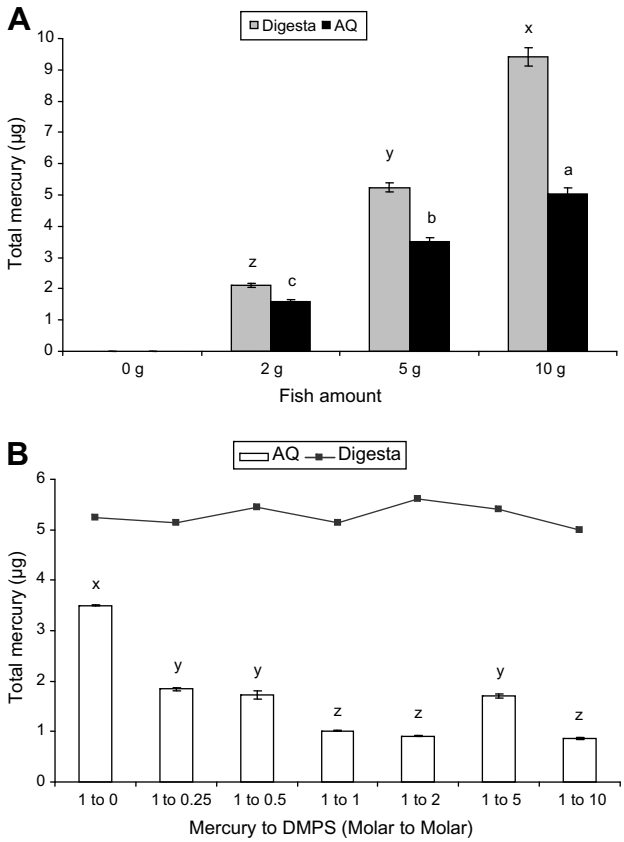

Fig. 1. Total mercury in digesta and aqueous phase (AQ) following *in vitro* digestion of fish tissue (A) and digestion of 5 g fish tissue (5 μg mercury) with DMPS (B). Digesta includes both the AQ and suspended solids. AQ includes liquid that was decanted from pellet. Values represent means ± standard error for 3 replications. Different letters (xyz or abc) indicate significance at the $\alpha = 0.05$ level.

2.3. In vitro digestion

Representative aliquots of each test meal were subjected to a simulated gastric and small intestinal digestion as described by Garret et al. (1999) and Ferruzzi et al. (2002). For the gastric phase of digestion, porcine pepsin (3 mg/ml, Sigma Chemical Co., St. Louis, MO) was added to samples, followed by acidification to pH 2 with 0.1 M HCl (Analytical grade, Sigma Chemical Co.). Samples were incubated at 37 °C for 1 h in a shaking water bath at 150 rpm (VWR, Cornelius, OR). To mimic digestion in the small intestine, the gastric digest was neutralized to pH 5.3 by addition of 100 mM sodium bicarbonate solution (Sigma Chemical Co.); then 9 ml of bile extract/pancreatin/lipase mixture: pancreatin (0.4 mg/ml, Sigma Chemical Co., St. Louis, MO), lipase (0.2 mg/ml, Sigma Chemical Co.) and porcine bile extract (2.4 mg/ml, Sigma Chemical Co.) were added. Sample pH was adjusted to 7.0 ± 0.5 using 0.1 M NaOH (Analytical grade, Sigma Chemical Co.). Samples were incubated in a shaking water bath for 2 h at 37 °C, 150 rpm, after flushing the top of the tubes with nitrogen gas (99.99%, Air Gas, Indianapolis, IN). Aliquots of the final digest (30 ml) were centrifuged at 167,000 *g* for 35 min (Beckman L8-70M, Beckman Coulter, San Antonio, TX), in order to isolate the aqueous phase from the particulate residue. Aliquots of raw material, digesta, aqueous phases, and residual pellets were collected and stored at −80 °C prior to analysis.

2.4. Determination of mercury

An aliquot of raw material, digesta, aqueous phases, and residual pellets was analyzed for total mercury using a Thermal Decomposition (Gold) Amalgamation/Atomic Absorption Spectrophotometer (TDA/AAS) (DMA-80 Mercury Analyzer, Milestone Inc., Pittsburgh, PA), as described by Shim, Dorworth, Lasrado, Santerre et al. (2004). Certified Reference Materials (CRMs: Tort-2 and Dorm-2, Institute for Environmental Chemistry, National Research Council Canada, Ottawa, Canada) were used to standardize the instrument. Total mercury concentrations in phytochemical-rich foods/ingredients were below the limit of detection (0.01 ng total mercury). Mercury bioaccessibility from fish test meals was defined as the fraction of mercury transferred from the fish matrix to the aqueous phase during *in vitro* digestion. Mercury recovery, which was determined by comparing the amount of mercury added in the fish tissue to the amount of mercury measured in the digesta, ranged from 94% to 108%. Bioaccessibility of mercury from fish test meals (0–10 g fish) without added food/food component was assessed.

2.5. Statistical analysis

Results are presented as representative data from triplicate sets of experiments. Data are expressed as means ± standard error of mean. Statistical analysis for each parameter assessed was performed by using analysis of variance (ANOVA), followed by Tukey's *post-hoc* test (SAS, Gary, NC.). Differences among means were considered statistically significant at $p < 0.05$.

3. Results and discussion

The amount of mercury transferred to the aqueous phase increased in a linear manner as the amount of fish tissue was increased, i.e., the bioaccessibility, from 2 g (2 μg Hg), 5 g (5 μg Hg), and 10 g (10 μg Hg) (Fig. 1A).

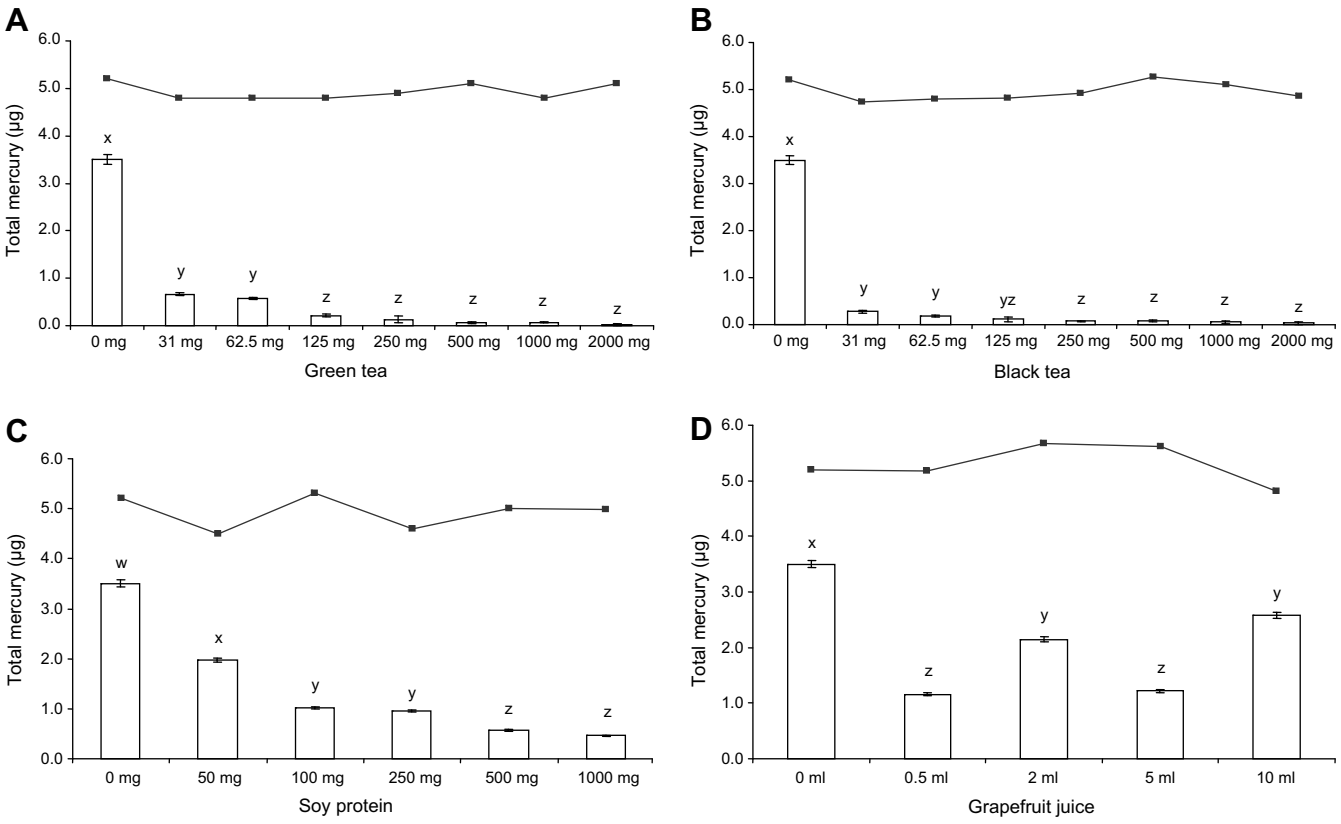

Fig. 2. Total mercury in digesta (■) and aqueous phase (▨) following *in vitro* digestion of 5 g fish tissue (5 μg mercury) in the presence of increasing amounts of green tea (A), black tea (B), soy protein (C) and grapefruit juice (D). Digesta indicates both aqueous phase and pellets. Digesta includes both the AQ and suspended solids. AQ includes liquid that was decanted from pellet. Values represent means ± standard error for 3 replications. Different letters (xyz or abc) indicate significance at the $\alpha = 0.05$ level.

The effects of DMPS on the concentration of mercury in the aqueous phase and the digesta (aqueous phase plus suspended solids) are shown in Fig. 1B. DMPS significantly ($p < 0.05$) reduced mercury bioaccessibility from fish tissue in a dose-dependent fashion (from 1:0.25 to 1:2). 1:1, 1:2 and 1:10 molar ratios of Hg to DMPS were most effective for reducing mercury bioaccessibility by 48–52%.

Phytochemical-rich foods, i.e., green tea, black tea, soy protein, and grapefruit juice, had a significant impact on mercury bioaccessibility (Fig. 2). In all cases, the presence of the foods served to reduce mercury bioaccessibility from the fish tissue. Green tea and black tea appeared to be most effective at lowering mercury in the aqueous phase at lower amounts, such as 30 and 62.5 mg (Fig. 2A–B). Soy protein significantly reduced mercury bioaccessibility in a dose-dependent manner (Fig. 2C). Flavonoids-rich grapefruit juice also resulted in a significant reduction in mercury bioaccessibility; however, higher grapefruit juice amount did not cause a greater reduction in mercury bioaccessibility (Fig. 2D).

Dietary fibres were also found to modulate mercury bioaccessibility (Fig. 3A–C). Wheat bran had the greatest effect on mercury bioaccessibility (72-84% reduction). Oat bran and psyllium only caused a significant reduction in mercury bioaccessibility in amounts higher than 100 mg (59–75% and 15–31% for oat bran and psyllium, respectively).

In this study, a two-stage *in vitro* digestion method was utilized to evaluate the bioaccessibility of mercury from fish tissue as modulated by the presence of foods containing phytochemicals and dietary fibres. Fish tissue and added phytochemicals/dietary fibres were subjected to both gastric and small intestinal stages of digestion. Following digestion, the resulting digesta were centrifuged to separate the suspended solids from the aqueous phase. Mercury extracted from the food matrix and transferred (solubilized) to the aqueous phase during digestion is presumed to be available (bioaccessible) for subsequent absorption by absorptive epithelial cells of the small intestine. This observation implies that mercury in the solid phase may not be bioaccessible but could be resolubilized in the lower intestine. The effects of food containing phytochemicals or dietary fibre on *in vitro* digestive stability of mercury were investigated by co-digestion of fish tissue with treatments (DMPS, green tea, black tea, soy protein, or grapefruit juice). The digestive environment allows for physiologically significant interaction between mercury from the fish tissue and phytochemicals. Green and black tea caused a proportional increase of mercury in the non-digestible residue (which was isolated as a pellet following centrifugation, data not shown). Phytochemicals present in tea, including catechins (green tea) and theaflavins and thearubegins (black tea) are well known chelators of metals (Chapman & Chan, 2000). It is plausible that these phytochemicals may form insoluble complexes with mercury and reduce bioaccessibility. Isolated soy protein, and wheat bran also decreased bioaccessibility of mercury from fish tissue following *in vitro* digestion. These findings were also found by Hojbjerg (1996) who reported that high protein levels in foods significantly decreased retention of both organic and inorganic mercury after oral exposure. The unexpected finding that grapefruit juice had little effect on reduction of mercury may be due to the fact that interactions between mercury and phytochemicals might be disrupted by other constituents (sugar, vitamin C and citric acid) in grapefruit juice.

Wheat bran significantly reduced mercury bioaccessibility which is consistent with the findings of Rowland et al. (1986), who reported that wheat bran was a scavenger of mercury, cadmium and lead. On the other hand, psyllium did not have a significant effect on mercury bioaccessibility, most likely due to the fact that psyllium contains more soluble than insoluble fibre, as shown in Table 3. We therefore hypothesize that insoluble fibre has a stronger mercury-binding capacity than has soluble fibre.

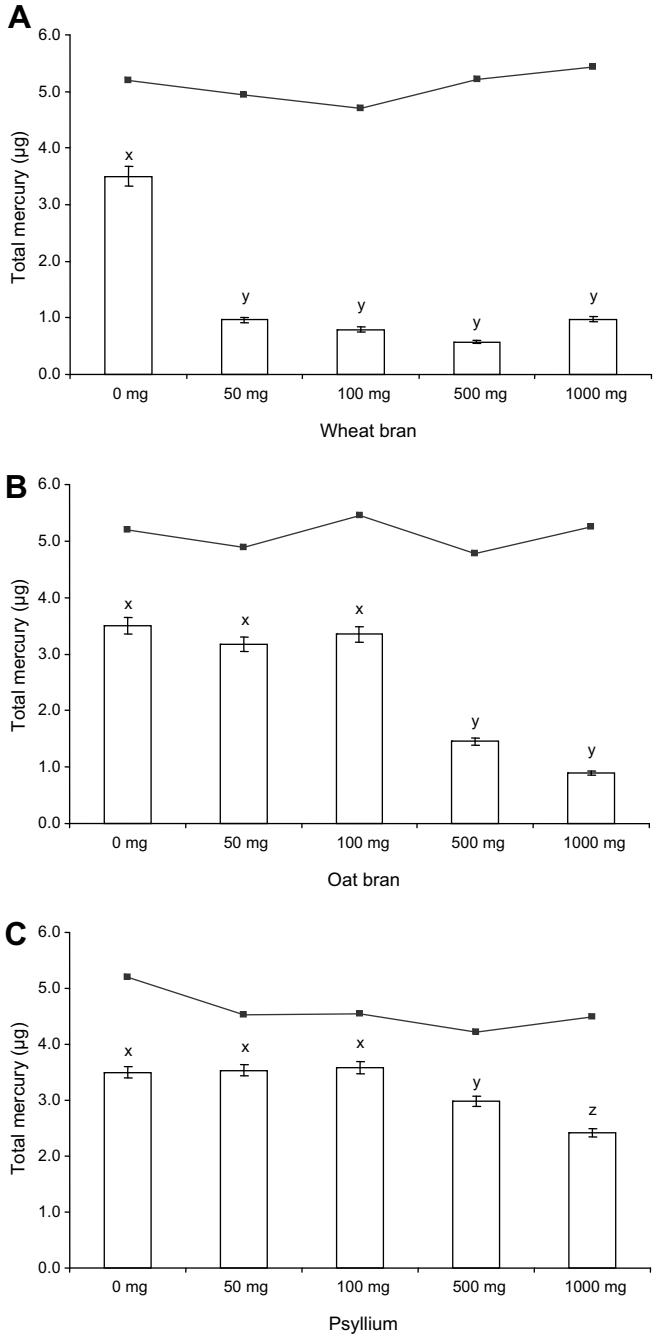

Fig. 3. Total mercury in digesta (■) and aqueous phase (▒) from *in vitro* digestion of 5 g fish tissue (5 µg mercury) in the presence of increasing amounts of wheat bran (A), oat bran (B) and psyllium (C). Digesta includes both the AQ and suspended solids. AQ includes liquid that was decanted from pellet. Values represent means ± standard error for 3 replications. Different letters (xyz or abc) indicate significance at the $\alpha = 0.05$ level.

Table 3
Composition of dietary fibres in oat bran, hard red wheat bran and psyllium

	Oat bran[a] (%)	Hard red wheat bran[a] (%)	Psyllium[b] (%)
Total dietary fibre	18.2	49.7	100
Soluble fibre	6.3	2.80	70
Insoluble fibre	11.9	46.9	30

[a] Data from American Association of Cereal Chemists (AACC, MN. USA).
[b] Data from, Proctor & Gamble Company (OH, USA).

In conclusion, this study supports the positive effects of foods containing phytochemicals or dietary fibre on the inhibition of digestive mercury stability when evaluated using an *in vitro* digestion model. Therefore, these results suggest that food which is rich in phytochemicals may be as efficient as synthetic chelating agents (e.g., DMPS) for long-term chronic methylmercury exposure in fish-eating populations by reducing mercury bioavailability. In addition, the benefits of the simulated *in vitro* digestion techniques described in this study relate to a system that may provide a rapid and cost-effective alternative for both evaluating bioaccessibility of mercury and screening food component effects on mercury bioaccessibility. However, in order to confirm the correlation between mercury bioaccessibility and cellular mercury uptake, further studies, combining a human intestinal cell model with testing in an *in vivo* system, are required.

Acknowledgements

The authors wish to thank Drs. Jon Story and Connie Weaver in the Department of Foods and Nutrition at Purdue University, Mr. George Henderson in the Florida Department of Environmental Protection at Tallahassee, and P&G for dietary fibre, soy protein composition, king mackerel, and psyllium, respectively.

References

Allen, K. G., & Harris, M. A. (2001). The role of $n-3$ fatty acids in gestation and parturition. *Experimental Biological Medicine, 226*, 498–506.

Aposhian, H. U., Maiorino, R. M., Rivera, M., Bruce, D. C., Dau, R. C., Hurlbut, K. M., et al. (1992). Human studies with the chelating agents; DMPS and DMSA. *Clinical Toxicology, 30*(4), 505–528.

Cha, C. W. (1987). A study on the effect of garlic to the heavy metal poisoning of rat. *J. Korean Med. Sci., 2*, 213–223.

Chapman, L., & Chan, H. M. (2000). The influence of nutrition on methylmercury intoxication. *Environmental Health Perspects, 108*(1), 29–56.

Ferruzzi, M. G., Failla, M. L., & Schwartz, S. J. (2001). Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and Caco-2 human cell model. *Journal of Agricultural and Food Chemistry, 49*, 2082–2089.

Ferrruzzi, M. G., Failla, M. L., & Schwartz, S. J. (2002). Sodium copper chlorophyllin: *in vitro* digestive stability and accumulation by Caco-2 human intestinal cells. *Journal of Agricultural and Food Chemistry, 50*, 2173–2179.

Garrett, D. A., Failla, M. L., & Sarama, R. J. (1999). Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. *Journal of Agricultural and Food Chemistry, 47*, 4301–4309.

Glahn, R. P., Lee, O. A., Yeung, A., Goldman, M. I., Miller, D. D., et al. (1998). Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model. *Journal of Nutrition, 128*, 1555–1561.

Hojbjerg, S. G. (1996). The effect of nutritional factors on absorption, retention and excretion or organic and inorganic mercury in mice and rats (abstract). *Danish Medical Bulletin, 43*(4), 376.

Lauritzen, L., Hansen, H. S., Jorgensen, M. H., Michaelsen, K. F., et al. (2001). The essentiality of long chain $n-3$ fatty acids in relation to development and function of the brain and retina. *Progress in Lipids and Research, 40*, 1–94.

Liu, C. S., Glahn, R. P., & Liu, R. H. (2004). Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an *in vitro* digestion. *Journal of Agricultural and Food Chemistry, 52*, 4330–4337.

Marsh, D., Clarkson, T. W., Myers, G. J., Davidson, P. W., Cox, C., Cernichiari, E., et al. (1995). The Seychelles study of methylmercury exposure and child development: Introduction. *Neurotoxic, 16*, 583–596.

Minamisawa, M., Minamisawa, H., Yoshida, S., Takai, N., et al. (2004). Adsorption behavior of heavy metals on biomaterials. *Journal of Agricultural and Food Chemistry, 52*, 5601–5611.

NAS. 2000. Toxicological Effects of Methyl Mercury. Washington, DC: national Academy Press (Chapter 2 & 3, pp. 31–95).

Oomen, A. G., Rompleberg, C. J. M., Bruil, M. A., Dobbe, C. J. G., Pereboom, D. P. K. H., Sips, A. J. A. M., et al. (2003). Development of an *in vitro* digestion model for estimation of bioaccessibility of soil contaminants. *Archives of Environment Contamination and Toxicology, 44*, 281–287.

Ou, S., Gao, Kongrong., & Li, Yan. (1999). An *in vitro* study of wheat bran binding capacity for Hg, Cd, and Pb. *Journal of Agricultural and Food Chemistry, 47*, 4714–4717.

Rhee, M. G., Cha, C. W., & Bae, E. S. (1985). The chronological changes of rat tissue and the effect of garlic in acute methylmercury poisoning. *Korean University of Medical Journal, 22*(1), 153–164.

Rogers, I., Emmett, P., Ness, A., Golding, J., et al. (2004). Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. *Journal of Epidemiology of Communication Health, 58*, 486–492.

Rowland, I. R., Mallett, A. K., Flynn, J., Hargreaves, R. J., et al. (1986). The effect of various dietary fibers on tissue concentration and chemical form of mercury after methylmercury exposure in mice. *Archive of Toxicology, 59*, 94–98.

Shim, S. M., Dorworth, L. E., Lasrado, J. A., Santerre, C. R., et al. (2004). Mercury and fatty acids in canned tuna, salmon, and mackerel. *Journal of Food Science, 69*(9), 681–684.

US EPA (US Environmental Protection Agency). 1997. Mercury Study Report to Congress (Vol. 1) Executive Summary. Washington, DC: Environmental Protection Agency; Publication EPA-452/R-97-003.

US FDA (US Food and Drug Administration). (2004). FDA and EPA announce the revised consumer advisory on methylmercury in fish. Food and Drug Administration. Rockville, Md. Available at http://www.fda.gov/bbs/news/2004/NEW01038.html. Accessed July 2004.

Versantvoort, C.H.M., Kamp, E.V.D., & Rompelberg, C.J.M. (2004). Development and applicability of an *in vitro* digestion model in assessing the bio accessibility of contaminants from food. RIVM rapport 320102002. Available from http://www.rivm.nl/bibliotheek/rapporten/320102002.pdf Accessed December 2004.

Versantvoort, C. H. M., Oomen, A. G., Kamp, E. V. D., Rompelberg, C. J. M., Sips, A. J. A. M., et al. (2005). Applicability of an *in vitro* digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology, 43*, 31–40.