Ecotoxicology and Environmental Safety 208 (2021) 111569

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Influence of nutrients on the bioaccessibility and transepithelial transport of polybrominated diphenyl ethers measured using an in vitro method and Caco-2 cell monolayers

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ARTICLE INFO

Edited by: Kurunthachalam Kannan

Keywords:
Bioaccessibility
Caco-2 cell monolayer
In vitro digestion
Nutrient
Transepithelial transport

ABSTRACT

Previous research has shown the absorption of polybrominated diphenyl ethers (PBDEs) in the human gastrointestinal tract, but limited attention has been given to the influence of nutrients on PBDE absorption from food matrices. We investigated the effects of nutrients (oil, starch, protein, and dietary fiber) on the absorption and transport of PBDEs in a Caco-2 cell model and bioaccessibility of PBDEs by an in vitro gastrointestinal digestion method. The results showed that the accumulation ratios of PBDE congeners in Caco-2 cells were higher in the nutrient addition groups (oil: 26.7–50.6%, starch: 27.0–58.7%, protein: 12.1–44.1%, and dietary fiber: 28.2–55.1%) than the control group (7.17–36.1%), whereas the transport ratios were lower (oil: 2.30–7.20%, starch: 1.55–9.15%, protein: 1.04–8.78%, and dietary fiber: 0.85–7.04%) than control group (3.78–11.1%). Additionally, the PBDE bioaccessibility could be increased by adding the nutrients, particularly oil and starch. This study clarified the differences in PBDE absorption in the presence of nutrients using the in vitro digestion and Caco-2 cell model. The findings showed that nutrients were an important factor that promoted PBDE absorption in the gastrointestinal tract. Therefore, it is important to focus on a novel dietary strategy of food consumption with contaminant compounds to protect human health.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as additives in consumer and industrial products because of their excellent flame retardant properties (Ohajinwa et al., 2019). There are three available technical mixtures, i.e., Penta-BDEs, Octa-BDEs, and Deca-BDE. These compounds have been added to the Stockholm Convention as priority controlled persistent organic pollutants because of their environmental persistence, bioaccumulative properties in biota, and toxicity to organisms (Pardo et al., 2020; Salamova et al., 2014). Although several PBDEs are banned worldwide, Deca-BDE is still used in China and can easily be metabolized into lower brominated congeners (Huwe and Smith, 2007; W.L. Li et al., 2016; Y.Y. Li et al., 2016). Thus, PBDEs have been ubiquitously detected in various environment matrices, such as air, water, soil and sediment, and introduced into the food chain (Genuis et al., 2017; L. Li et al., 2020; H.W. Li et al., 2020; Percy et al., 2020; Wang et al., 2020; Windsor et al., 2020). Human exposure to PBDEs primarily occurs through dietary ingestion, whereupon the chemicals become stored in human tissue, acting as sources of long-term internal exposure (Domingo, 2012; Qin et al., 2019).

For human exposure assessment, the oral bioaccessibility of contaminants is generally measured by using an in vitro method simulating the human digestion process. It is defined as the fraction of contaminants released from a matrix into the digestion solution, and it reflects the maximum possible absorption fraction in the human gastrointestinal tract (Dean and Ma, 2007; Yu et al., 2011). A Caco-2 cell (originating from a human colon adenocarcinoma) monolayer is an excellent tool for assessing the absorption of substances in the intestine. This type of

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https://doi.org/10.1016/j.ecoenv.2020.111569
Received 18 September 2020; Received in revised form 20 October 2020; Accepted 25 October 2020
Available online 10 November 2020

monolayer displays many functional characteristics of enterocytes, including an apical membrane with a microvilli brush border and basolateral membrane, which both express various protein transporters. Thus, it is a well-developed model for exploring the carrier-mediated transport and intracellular accumulation mechanisms of nutrients in the human intestine (Braga et al., 2018; Guerra et al., 2012; Takenaka et al., 2015; Ting et al., 2015). Therefore, both an in vitro method and Caco-2 cell monolayer are suitable approaches for evaluating the human oral ingestion of PBDEs.

Food has been identified as the main potential source of human exposure to PBDEs. Generally, food components can alter the absorptive capacity of target compounds in the human digestive system, especially lipids, proteins and some carbohydrates. For example, dietary lipid, a well-known nutrient, can promote the bioaccessibility of target compounds in the gastrointestinal tract. The more unsaturated fatty acids are, the easier they form larger micelles, which have been shown to improve the absorption of pterostilbene in simulated gastrointestinal experiments (Kopec and Failla, 2018; Liu et al., 2019; Victoria-Campos et al., 2015). Pectin can easily combined with the medium, which reduces the micellization formation of carotenoid in the medium, in end, carotenoid dispersed into cells. Chitosan, like pectin, a kind of polysaccharide, has been demonstrated to significantly increase curcumin bioaccessibility in a dynamic gastrointestinal model and transport of curcumin through Caco-2 cells (Chitchumroonchokchai et al., 2004; Silva et al., 2019). These findings suggest that nutrients could potentially affect the absorption of target compounds in the gastrointestinal system.

Dietary ingestion of PBDEs may pose great risks to human health. However, the effects of nutrients on PBDE absorption have not been adequately characterized due to the complexity of the human digestive system. Therefore, in the present study, we utilized an in vitro method and Caco-2 cell monolayers to provide insights into the effects of nutrients on the digestion and absorption of PBDEs in the human gastrointestinal tract and verify the applicability of in vitro experiments. An understanding of the transport properties and factors influencing PBDE absorption in the human digestive system is crucial and can provide information for designing effective strategies to protect humans from the exposure risks of PBDEs.

2. Materials and methods

2.1. Materials and reagents

PBDE standards consisting of BDE28, 47, 66, 85, 99, 100, 153 and 183 were obtained from AccuStandard (CT, USA). The surrogate standard ^{13}C-PCB141 and internal standard ^{13}C-PCB208 were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Mucin from porcine stomach (type Π), bovine bile, D-(+)-glucose, and starch from potato were obtained from Sigma-Aldrich (St. Louis. MO, USA). Yeast extract, pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, and peptone from poultry were obtained from Merck (Darmstadt, Germany). Xylan from birch wood, pectin from apples, and (+)-arabinogalactan from larch wood were purchased from Fluka (New Jersey, USA). Acetone, dichloromethane, *n*-hexane and other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Caco-2 cells was purchased from BioHermes Bio & Medical Technology Co., Inc. (Wuxi, China). Fetal bovine serum and minimum essential medium (MEM) were purchased from Thermo Scientific Co., Inc. (San Jose, CA, USA). Twelve-well Transwell inserts (pore size, 0.4 μm, diameter, 12 mm) were purchased from Corning Co., Inc. (New York, USA), and 96-well plates were obtained from Fisher Scientific (Houston, TX, USA). Penicillin/streptomycin (10 mg/mL) was obtained from Invitrogen Life Technologies Inc (Burlington, ON, Canada). A 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) solution was obtained from Sigma-Aldrich (St. Louis. MO, USA). An epithelial voltammeter (EVOM2) was obtained from World Precision Instruments (Sarasota, FL, USA). Silica gel (80−100 mesh) was purchased from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China). NaCl, KCl, KH_2PO_4, $Na_2HPO_4 \cdot 12\ H_2O$ and $NaHCO_3$ were purchased Anpel (Shanghai, China).

Digestion solutions were prepared as described in our previous report (Yu et al., 2009). Briefly, a solution of gastric acid was prepared by dissolving 0.09 g of pepsin from porcine gastric mucosa in 1 L of 0.1 mol/L HCl, and then it was sterilized using a 0.22 μm filter. The nutrient solution consisted of mucin from porcine stomach (type Π) (4.0 g/L), yeast extract (3.0 g/L), (+)-arabinogalactan from larch wood (1.0 g/L), pectin from apples (2.0 g/L), xylan from birch wood (1.0 g/L), L-aminothiopropionic acid (0.5 g/L), D-(+)-glucose (0.4 g/L), peptone from poultry (1.0 g/L) and starch from potato (3.0 g/L). The gastric juice was prepared from the gastric acid solution and nutrient solution in a volume ratio of 1:8 with the pH value adjusted to 3.0. The intestinal solution consisted of Na_2HPO_4 (12.5 g/L), bovine bile (0.6 g/L) and pancreatin from porcine pancreas (0.9 g/L). The solutions were stored at 4 °C before use. Additionally, the salt solution D′Hank was used to rinse the cells, and it was prepared by dissolved 8.0 g NaCl, 0.4 g KCl, 0.06 g KH_2PO_4, 0.08 g $Na_2HPO_4 \cdot 12H_2O$ and 0.35 g $NaHCO_3$ in 1000 mL ultrapure water. After mixed, hydrochloric acid adjusts the pH value to 7.0–7.4, autoclave sterilized (121 °C), and store for use.

2.2. Cell culture and cell viability assay

Caco-2 cells were cultured in MEM containing 1% penicillin/streptomycin and 20% fetal bovine serum. They were incubated under a humidified incubator with 5% CO_2 at 37 °C. When the cell monolayer reached 80% confluence, the cells were passaged with 0.25% trypsin-EDTA. The culture medium was replaced every 1–2 d.

To assess the effects of PBDEs, digestive juice and nutrients on Caco-2 cells, the cytotoxicity was analyzed by a MTT (3-(4,5-Dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide) assay as described previously (Yu et al., 2017). Briefly, after Caco-2 cells were harvested, they were seeded in a 96-well plate at a density of 3000 cells per well in 100 μL medium. After incubation for 24 h, they were exposed to the target substances (PBDEs, digestive juice, or nutrients) at different concentrations for 12 h, then the medium containing the targets was replaced with 100 μL 0.25% mg/mL MTT solution and the cells were incubated for a further 4 h. Afterwards, 150 μL DMSO was added to the wells to dissolve any dark blue formazan crystals formed due to reaction with NAD(P)H-dependent cellular oxidoreductase enzymes in viable cells. The absorbance, expressed as % cell viability, was determined at 490 nm using an iMark plate reader. Each experiment was repeated six times. Detailed exposure concentration information is shown in Table 1.

Table 1
Exposure concentrations of PBDEs (ng/mL), digestive juice and nutrients (g) in the MTT assay.

PBDEs	Digestive juices	Oil	Starch	Protein	Dietary fiber
0	CON	0.01	0.01	0.15	0.01
2.5	1/5CON	0.05	0.05	0.2	0.04
5	1/10CON	0.2	0.2	0.3	0.08
10	1/15CON	0.5	0.5	0.5	0.15
20	1/20CON	1	1	1	0.3
–	1/40CON	2	–	1.5	–

Note: CON represents the control with the original standard digestive juice concentration, 1/5 CON represents the original digestive juice diluted five times, etc. After MTT validation experiments, the exposure conditions for the bioaccessibility evaluation included the nutrients oil, starch, protein or dietary fiber. Oil: 0.001, 0.02, and 0.08 g sunflower seed oil; starch: 0.001, 0.05, and 0.1 g starch; protein: an egg was cooked, the egg white dried, ground into powder and 0.001, 0.02, and 0.1 g used; dietary fiber: 0.001, 0.01, and 0.02 g pectin.

2.3. Transport experiments using a Caco-2 cell monolayer

Experiments investigating the effect of nutrients on PBDE transport across a Caco-2 cell monolayer were carried out as follows. Caco-2 cells were cultured in 12-well inserts at an initial density of 1×10^5 cells/cm^2 for 21 d. Formation of the Caco-2 cell monolayer was indicated by a transepithelial electrical resistance (TEER) >400 Ω/cm^2 measured with an epithelial voltammeter. After formation, the cell monolayer was gently rinsed twice with D′Hanks, and the volumes of medium in the apical (AP) and basolateral (BL) chamber were maintained at 0.5 and 1.5 mL, respectively. The cells were exposed to 10 ng/mL PBDEs for 12 h in the AP side with nutrients, i.e., oil (0.01, 0.2 or 1.0 g), starch (0.01, 0.5 or 1.0 g), protein (0.15, 0.3 or 1.5 g), or dietary fiber (0.01, 0.15 or 0.3 g). And then, the medium containing PBDEs in the AP and BL side were collected and gently rinsed with D′Hanks completely (6 mL in total per sample). It was used for further treatment.

PBDEs in medium collected from the AP or BL side in the transport experiments were measured as follows. After spiking the solutions with the surrogate standard ^{13}C-PCB141, PBDEs were extracted with a mixture of 6 mL acetone, 3 mL *n*-hexane and 9 mL dichloromethane three times, as described in a previous study (Yu et al., 2017). The combined organic phase was concentrated and cleaned up through a silica-alumina column with 70 mL mixture of dichloromethane and *n*-hexane (1:1, v/v). The eluent containing PBDEs was concentrated to 1 mL under a gentle stream of nitrogen. After spiking with the internal standard ^{13}C-PCB208, the final sample was stored in 50 μL isooctane at −20 °C until gas chromatography/mass spectrometry (GC/MS) analysis.

To measure the intracellular accumulation of PBDEs, cells were harvested from the Transwell inserts by incubating them with 500 μL trypsin for 4 h, followed by rinsing with HBSS (6 mL in total per sample) and disrupting them with cell breaking apparatus (XL-2000, ultrasonic vibration at 3 Hz for 1 min). The resulting lysate was extracted by liquid-liquid extraction as described above.

2.4. In vitro method for measuring the bioaccessibility of PBDEs

To investigate the influence of nutrients on the bioaccessibility of PBDEs, a fish sample was used with concentrations of 197, 12.6, 148, 93.3, 179, 23.1, 109 and 201 ng/g dry weight of BDE28, 47, 66, 85, 99, 100, 153 and 183, respectively, which determined as previous method (Yu et al., 2012a). Generally, 0.2 g fish sample and nutrients (of known amount) were added to 12 mL simulated gastric juice and incubated at 37 °C for 2 h. Next, 6 mL simulated intestinal digestive fluid was added and further incubated for 6 h. Afterwards, the digested solution was centrifuged at 7000×*g* at room temperature for 10 min. The supernatant was filtered through a 0.45 μm polypropylene fiber membrane and then extracted for PBDE measurement.

PBDEs in digestive juices were determined by a method reported previously with modifications (Yu et al., 2012b). A surrogate standard ^{13}C-PCB141 was added to 15 mL of the filtered digestive juices, and 20 mL acetone was added. PBDEs were extracted three times using 30 mL *n*-hexane:dichloromethane (1:3, v/v). The collected organic phase was concentrated to approximately 1 mL and then 30 mL *n*-hexane was added. Concentrated sulfuric acid (98%) was used to remove impurities, such as proteins and lipids. The organic layer was cleaned up through a multiple silica-alumina column with 70 mL *n*-hexane:dichloromethane (1:1, v/v). The eluent was concentrated and stored in 50 μL isooctane at −20 °C until GC/MS analysis after adding the internal standard ^{13}C-PCB208.

2.5. Instrumental analysis

The analysis of PBDEs was performed on an Agilent 6890 N gas chromatograph coupled with a 5975 mass spectrometer using the negative chemical ionization mode. A DB-5MS capillary column (30 m × 0.25 mm × 0.1 μm, J & W Scientific, USA) was used with helium as the carrier gas (1 mL/min). Splitless injection was used with a 1 μL sample. The temperatures of the injector and ion source were set at 280 and 250 °C, respectively. The oven temperature was held at 110 °C for 1 min, then increased to 300 °C at 10 °C/min, and finally held for 5 min. The selective ion monitoring mode was used and ions at m/z = 476/478, 372/374 and 79/81 were monitored for ^{13}C-PCB208, ^{13}C-PCB141 and tri- to hepta-BDE congeners, respectively.

2.6. Quality assurance and quality control

Procedural blanks were processed to monitor interfering peaks. Calibration plots for all the congeners had satisfactory linear regression coefficients ($R^2 > 0.99$). The method detection limit was 0.15−0.45 ng/g for tri- to hepta-BDE congeners. The reported concentrations were not corrected against the recoveries of the surrogate standard ^{13}C-PCB141 for the transport experiment (63.7 ± 11.5%) and in vitro method (86.1 ± 17.0%).

2.7. Calculations and statistical analyses

The transepithelial transport and intracellular accumulation of PBDEs in the Caco-2 cell monolayer was calculated using the following equations:

$$\mathrm{TR} = Q_t/Q_o \text{ and } \mathrm{AR} = Q_a/Q_o$$

where TR and AR (dimensionless) are the transported and accumulated ratios of a congener, respectively; Q_o, Q_t and Q_a (pg) are the total exposure dose of the congener in the AP side, the transported amount in the BL side and the accumulated amount of PBDEs in the cells, respectively.

The bioaccessibility (Ba%) of PBDEs was calculated according to the following equation:

$$\mathrm{Ba}(\%) = \frac{m_s}{m_t} \times 100\%$$

where m_s and m_t (pg) are the amounts of a congener released from the digestive juice (supernatant) and fish sample (total amount of the congener), respectively.

Statistical significance was analyzed by SPSS 13.0 software. Values of p less than 0.05 and 0.01 were considered to represent statistical significance and marginal significance, respectively.

3. Results and discussion

3.1. Cell viability after exposure to PBDEs and digestive medium

Caco-2 cell monolayers were used to simulate adsorption of PBDEs across the small intestine and effects of nutrients on the absorption. To carry out the transport experiment, the integrity of the Caco-2 cell model first had to be confirmed. Therefore, a MTT assay was conducted (Fig. 1). Fig. 1A shows the cytotoxicity assessment of PBDEs on Caco-2 cells. Over 90% of the cells were viable at all tested concentrations (2.5−20 ng/mL) compared to the control, indicating that PBDEs at these concentrations did not cause cytotoxicity to Caco-2 cells. Also, no obvious adverse effects on cell viability were observed for the digestive juices on Caco-2 cells over the tested concentration ranges (Fig. 1B). Thus, the results indicated that Caco-2 cell monolayers were a suitable model for conducting transport experiments of PBDEs under the tested concentrations.

Subsequently, we evaluated the effect of nutrients on Caco-2 cells (Fig. 1). After addition of 0.01−1 g oil, the cell viability was 85%−123% in digestive juice of concentration 0.07 CON (CON: control, represent the original standard digestive juice concentration) (Fig. 1C), indicating no markedly inhibitory or proliferative effect on Caco-2 cells. Similar results were observed for the other nutrients, i.e., starch, protein and

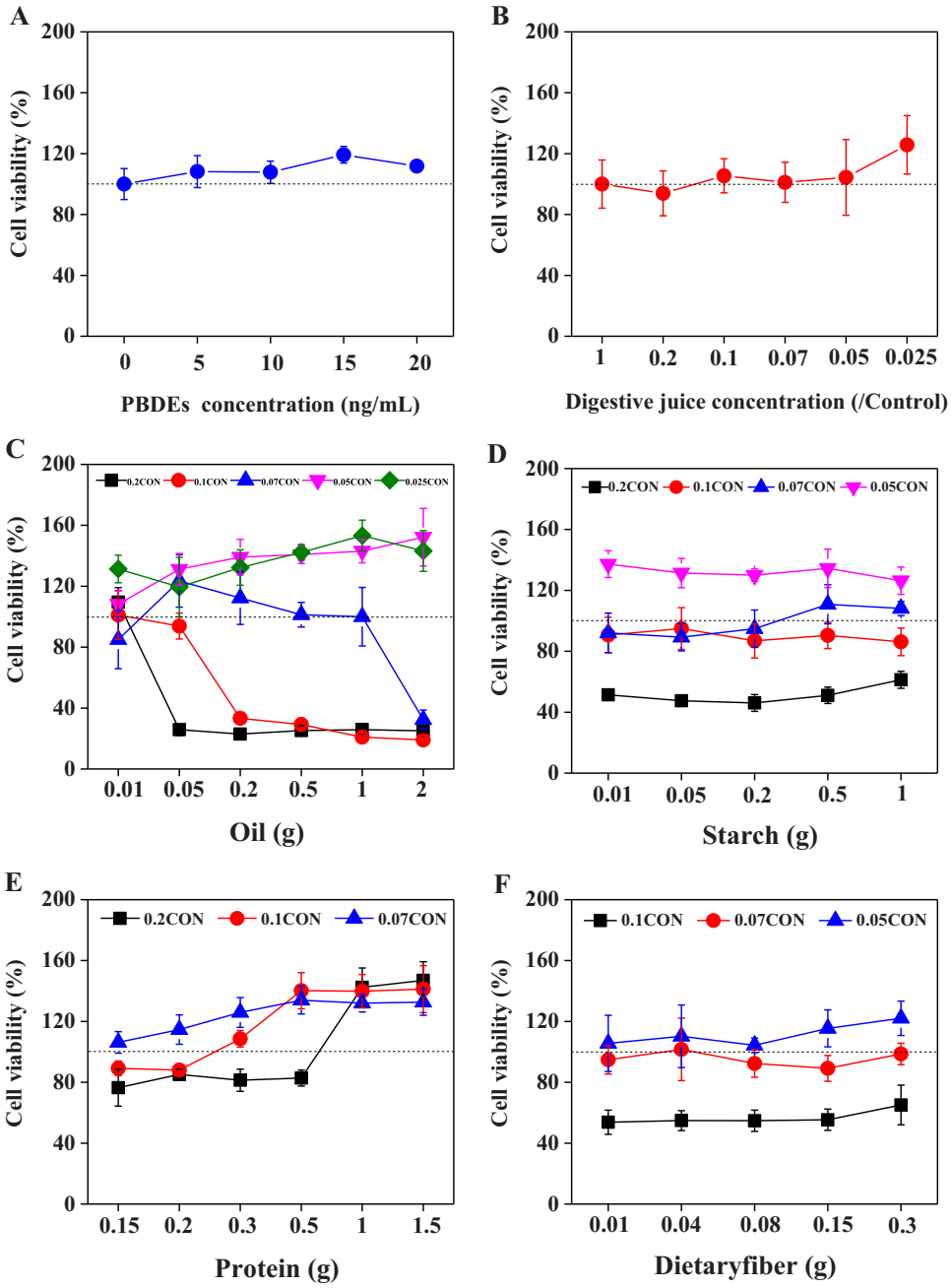

Fig. 1. Cell viability under different conditions. A: different concentrations of PBDEs; B: different concentrations of digestive juice; C: different amounts of oil in digestive juice; D: different amounts of starch in digestive juice; E: different amounts of protein in digestive juice; F: different amounts of dietary fiber in digestive juice. CON represents control samples with the original standard digestive juice concentration. The bars represent the standard deviation of parallel samples.

dietary fiber (Fig. 1D–F). The viabilities of Caco-2 cells were 86.2%–90.7%, 97.4%–133% and 89.2%–102% after the addition of 0.01–1.0 g starch (0.1 CON indicates the standard digestive juice diluted 10 times), 0.1–0.5 g protein (0.07 CON) and 0.01–3.0 g dietary fiber (0.07 CON), respectively. Thus, the results from the MTT assay demonstrated that the concentrations of PBDEs, gastrointestinal digestive juice and nutrients used for the transport investigation of PBDEs in the Caco-2 cell model did not cause any obvious cytotoxicity.

3.2. *Effects of nutrients on transepithelial transport of PBDEs*

To investigate the influence of nutrients on the absorption of PBDEs in the small intestine, the transported and accumulated PBDEs in Caco-2 cells were investigated (Fig. 2). It was found that a large number of PBDEs accumulated in Caco-2 cells (26.7%–50.6%) after oil addition (Fig. 2A), significantly higher than those of the control group (7.17%–36.1%) ($p < 0.01$). In contrast, most PBDEs exhibited significantly ($p < 0.01$) lower transport ratios (2.30%–7.20%) than the control group (3.78%–11.1%). However, there was no obvious difference in transport or accumulation ratios of PBDEs when adding different amounts of oil. The results indicated that oil effectively promoted the accumulation of PBDEs into Caco-2 cells, although the transepithelial transport ratios decreased. This can be explained by the fact that the oil was rich in unsaturated fatty acids, which may potentially alter the fatty acyl composition of phospholipids in the plasma membrane or activities of phase II enzymes in enterocytes, as well as increase the cell membrane permeability (Dominguez-Avila et al., 2017). The presence of co-ingested oil resembling lipids has been shown to promote secretion of

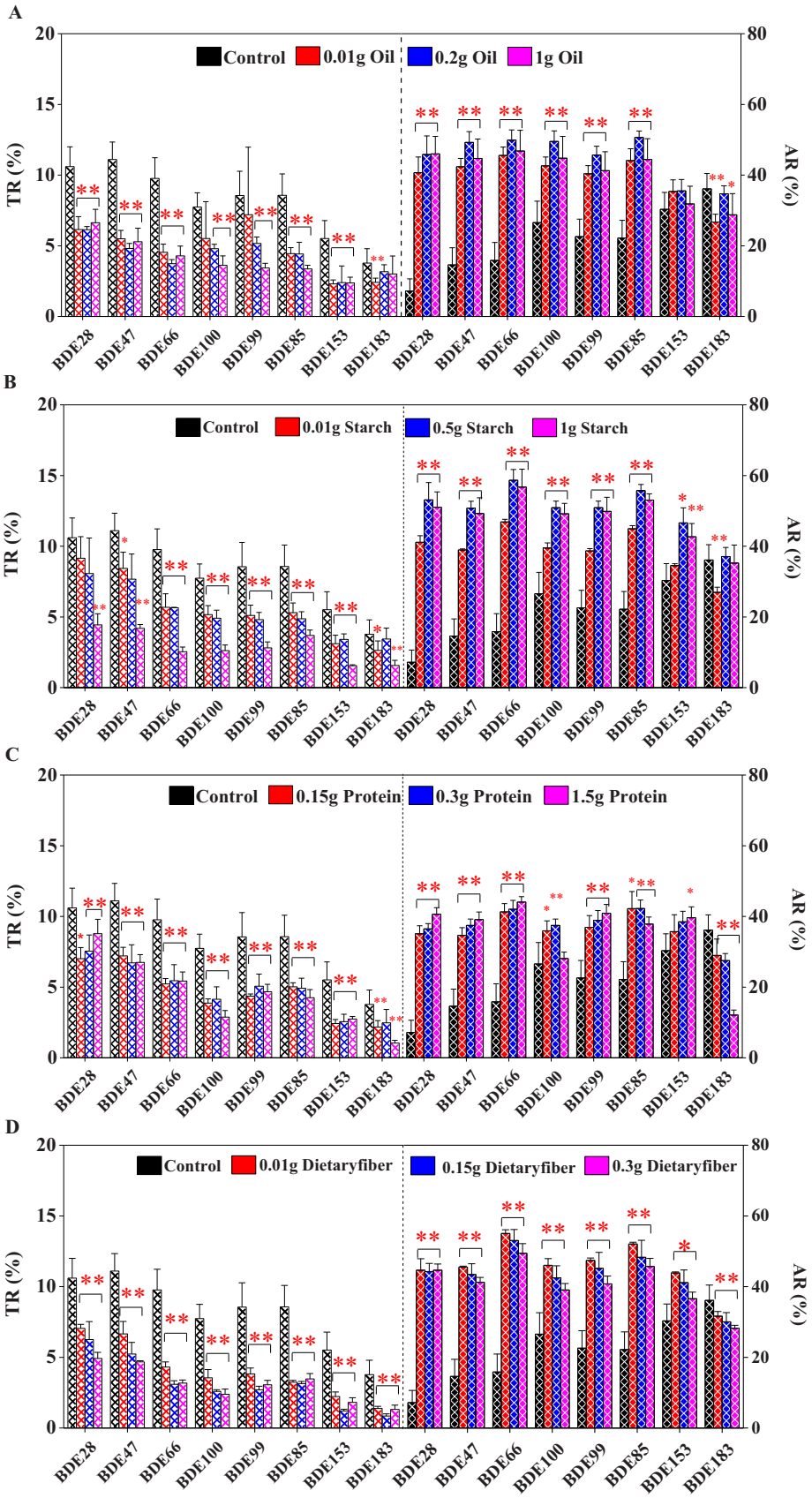

Fig. 2. Transported and accumulated ratios of PBDEs in the Caco-2 cell monolayer model treated with four nutrients (TR: transported ratio; AR: accumulated ratio. The error bars represent the standard deviation of parallel samples; star symbols indicate significant differences between the treatment group and control: $p < 0.05$, *; $p < 0.01$).

a large number of lipoprotein particles by stimulating the formation of chylomicrons within epithelium cells, increasing PBDE accumulation in cells (Failla et al., 2014). Additionally, unilateral exposure of cells to micelles combined with oil has been reported to enhance the absorption of lipophilic PBDEs, increasing the intracellular accumulation markedly, rather than being transported to the BL side as found for most PBDE congeners (Intawongse and Dean, 2006; Kotake-Nara and Nagao, 2012; Sun et al., 2015; Yang et al., 2017).

In the case of starch, a similar trend was observed (Fig. 2B). Generally, the accumulated ratios of PBDEs in the Caco-2 cells were 27.0%−46.9%, 37.1%−58.7% and 35.2%−56.7% after adding 0.01, 0.5 and 1 g of starch, respectively. With the exception of BDE183, these ratios were much higher than those of the controls (7.17%−36.1%) after adding 0.5 g or 1 g of starch ($p < 0.05$). However, the amounts of PBDEs transported to the BL side were still relatively low (1.55%−9.15%) and significantly lower than those of the control (3.78%−11.1%) ($p < 0.05$). Starch, a kind of polysaccharide, has been reported to alter the permeability of human endothelial monolayers without changing the cell vitality, increasing passive diffusion (Wong et al., 2019). Amylase bound to glycoprotein N-glycans in the brush-border membrane has been suggested to promote starch digestion while inhibiting glucose uptake (Date et al., 2015). In addition, hydrophobic interactions between lipid soluble compounds and amylopectin have been attributed to the transport and intracellular accumulation (Chen and Yao, 2017; Moser et al., 2016). Our results suggest that starch effectively enhances the intracellular accumulation of PBDEs and significantly reduces the transport in Caco-2 cells.

Regarding the effect of protein on the transport in the Caco-2 cells model, the accumulation of PBDEs increased significantly after the addition of protein only for the low molecular congeners, such as tri-, tetra- and penta-BDEs (Fig. 2C). The accumulation ratios were 28.1%–44.1%, higher than those of the controls (7.17%−26.5%). There was no obvious increase in the intracellular accumulation of the macromolecular congeners, although the underlying mechanism is still unknown. In contrast, the transported ratios (1.04%−8.78%) of most PBDEs showed a significant downward trend ($p < 0.01$), suggesting possible protein effects. This phenomenon has also been reported in the literature. For example, protein can be hydrolyzed into peptides, being bioactive able to promote calcium uptake via epithelium absorption. It directly attacked the targets proteins in the apical junctional complex causing the opening of the tight junction or serve as a carrier for paracellular transport of chemicals across the tight junction (Buaduang et al., 2019; Grootaert et al., 2017). In addition, the hydrogen bonds of proteins with hydrophobic interactions or polypeptides produced by proteolysis could significantly increase the accumulated ratios of compounds in Caco-2 cells (Henry, 2005; Maier-Salamon et al., 2006). Thus, in our study, PBDEs may have accumulated rapidly in Caco-2 cells, especially low molecular weight congeners, due to interactions between PBDEs and hydrogen bonds.

Similarly, there was a large increase in the intracellular accumulation of PBDEs in Caco-2 cells after dietary fiber added (36.6%−55.1%), except for BDE183 (Fig. 2D). In contrast, the transported ratios of PBDEs decreased significantly after dietary fiber addition compared with the controls (3.78%−11.1% vs. 0.85%−7.04%). Furthermore, the transported ratios of PBDEs decreased with increasing molecular weight, indicating that it was more difficult for the high brominated congeners to pass through the Caco-2 cell monolayer. Recently, several studies have reported that different dietary fibers are involved in the transport and absorption of compounds in Caco-2 cells (Kruger et al., 2020; Moser et al., 2020; Zhang et al., 2020). For example, dietary fibers in orange juice may potentially bind to flavonoids, preventing flavonoids from interfering with glucose transport (Moser et al., 2020). Also, as a food constituent, they can effectively change synthetic phenolic antioxidants in Caco-2 cells (Zhang et al., 2020). Pectin, a type of dietary fiber, has been reported to have inhibitory effects on Caco-2 cellular uptake of ferulic acid and naringenin (Kruger et al., 2020). In addition, the structural organization of the cell monolayer has been shown to be slightly affected (with no detectable TEER effect) by interactions of dietary fiber and protein, or the inhibition of the efflux transporter P-glycoprotein, leading to increased absorptive permeability (Kruger et al., 2020; Nart et al., 2015; Song and Chen, 2015; Tibolla et al., 2018). These may be important factors explaining the accumulation of PBDEs in Caco-2 cells in the present case.

3.3. Effect of nutrients on the Bioaccessibility of PBDEs

As mentioned above, nutrients such as oil, starch, protein and dietary fiber can influence the absorption of PBDEs in Caco-2 cells. To assess if there was also a correlation between the nutrients and bioaccessibility of PBDEs in fish samples, an in vitro method was used. The results are shown in Fig. 3. Oil from food was found to be the predominant factor affecting the bioaccessibility of PBDEs (Fig. 3A). The bioaccessibility clearly increased to 10.7%−23.9%, 16.4%−26.6% and 23.2%−35.0% after oil addition with 0.01, 0.2 and 1.0 g of oil, respectively. These results suggest that oil promotes the release of PBDEs from fish samples during digestion. Oil addition has been shown to favor micelle formation with bile salts, phospholipids, fatty acids and monoacylglycerols in a simulated human gastrointestinal tract (Yao et al., 2020). It also enhances the solubility of PBDEs in gastrointestinal fluids, thus increasing the bioaccessibility of PBDEs. In particular, it was shown that the bioaccessibility of lipophilic compounds was improved when lipophilic substances were co-ingested and encapsulated in small oil droplets (Gleize et al., 2013; Ornelas-Paz et al., 2008; Sy et al., 2012). These studies indicate that mixed micelles might be positively correlated with the concentration of lipid species in a digestion solution. As a result, the bioaccessibility of lipophilic bioactive compounds, such as carotenoids, can be increased (de Abreu-Martins et al., 2020; Singh et al., 2009). It has been reported that the bioaccessibility of curcumin increased from 11% to 28% in a bulk oil phase under a simulated intestinal digestion process (Lu et al., 2019).

As shown in Fig. 3B, the bioaccessibility of PBDEs ranged from 39.5% to 69.0% after starch was added, which were significantly higher values ($p < 0.01$) than for the controls (19.1%–34.0%). The results indicate that starch may also play a fundamental role in determining the bioaccessibility of PBDEs in fish samples. However, there was no significant difference in bioaccessibility among the different doses of starch. Starch, as a macromolecular carbohydrate, consists of amylose and highly branched amylopectin molecules. It can bind to compounds via hydrogen bonds, hydrophobic interactions, and electrostatic or ionic interactions, which may increase absorption in the gastrointestinal system (Chi et al., 2017; Dehghan-Shoar et al., 2011; Li et al., 2018; Limwachiranon et al., 2020). We suggest that PBDEs may bind to starch through a combination of hydrogen bonds and hydrophobic interactions, improving the bioaccessibility of PBDEs.

The influence of protein on the bioaccessibility of PBDEs was also observed (Fig. 3C). Generally, the bioaccessibility of most PBDE congeners increased significantly ($p < 0.05$) after protein addition, especially for the lowest doses (31.0%–44.4%). The effect of protein on the bioaccessibility was probably due to interactions between the PBDEs and protein, increasing the release of PBDEs from the matrix. It has been reported that β-lactoglobulin can increase the bioaccessibility of riboflavin and quercetin bioaccessibility (Simoes et al., 2020). Also, there is evidence suggesting that proteins or proteolytic products can increase the solubility of lipophilic compounds and increase the bioaccessibility of curcumin (H.W. Li and Yao, 2020; L. Li and Yao, 2020; Velasco-Reynold et al., 2008).

A similar phenomenon was observed regarding the effect of dietary fiber (Fig. 3D). However, only the lowest dose of dietary fiber resulted in a significant increase of the bioaccessibility of PBDEs to 24.0%−39.7% ($p < 0.05$), which were higher values than for the control group (19.1%−34.0%). At higher dietary fiber concentrations, the bioaccessibility decreased. Dietary fiber can bind to bile salts,

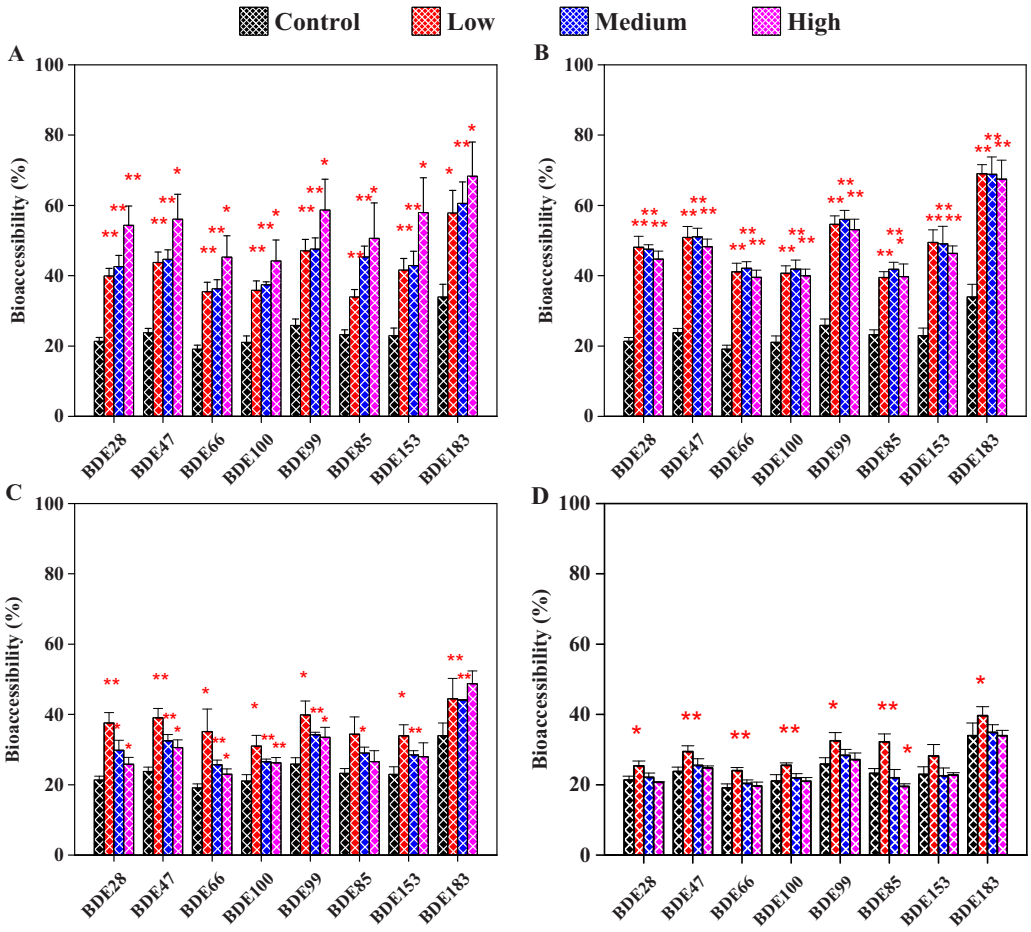

Fig. 3. Effects of nutrients on the bioaccessibility of PBDEs measured by the in vitro method (A: oil, B: starch, C: protein, D: dietary fiber). The error bars represent the standard deviation of parallel samples; star symbols indicate significant differences between the treatment group and control: $p < 0.05$, *; $p < 0.01$, **).

phospholipids, enzymes and compounds, increasing the viscosity of digestive juice, which may explain the bioaccessibility increase of PBDEs tested by the in vitro method (Chitchumroonchokchai et al., 2017). As described in other literature, dietary fiber could be used to prevent the absorption of toxic compounds by reducing their bioaccessibility in the gastrointestinal system. For example, dietary fiber such as pectin, methylcellulose, hydroxypropylmethylcellulose and carboxymethylcellulose from seafood could reduce mercury bioaccessibility subjected to gastrointestinal digestion (Piedra et al., 2016).

3.4. Comparison of the influential factors in the two models

To further understand the nutrient influence on the bioaccessibility of PBDEs in the in vitro and Caco-2 cell monolayer methods, the data were further analyzed (Fig. 4). Without any nutrient addition, the accumulation ratios of PBDEs by Caco-2 cell model were higher than the bioaccessibility measured using the in vitro method. For oil and starch, there was no significant difference between the data of PBDEs in the two methods: the values were 32.4%–75.0% in the in vitro method and 26.3%–57.9% in Caco-2 cells after oil was added, and 35.7%–75.6% in the in vitro method and 27.7%–66.8% in Caco-2 cells after starch was added. However, for the influences of protein and dietary fiber, the mean absorption ratios of PBDEs in Caco-2 cells (protein: 41.0%; dietary fiber: 46.7%) were generally higher than the bioaccessibility in the in vitro method (protein: 32.8%; dietary fiber: 26.0%). Therefore, the results indicated there were different degrees of influence of protein and dietary fiber on the bioaccessibility and accumulation ratio of PBDEs.

It should be noted that the present in vitro digestion method is a type

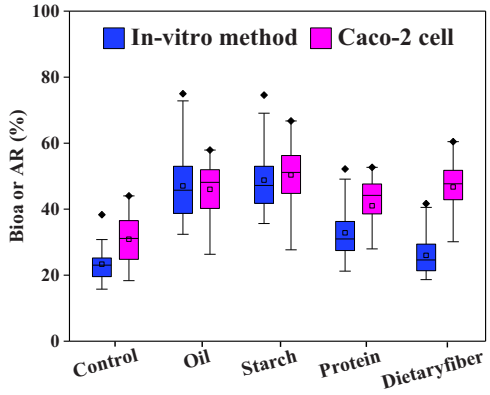

Fig. 4. Comparison of the bioaccessibility of PBDEs from the in vitro method and accumulation ratios of PBDEs in Caco-2 cells (Bioa: bioaccessibility; AR: accumulation ratio). The lower and upper limits of the box denote the 25th and 75th quartiles, respectively; the horizontal line within the box denotes the median value; the length of each whisker is 1.5 times the interquartile range; the rhombus symbol denotes the 99th percentile; small rectangular boxes represent average values.

of static model. After a certain period of incubation time, the release of PBDEs from the matrix (fish sample in the present case) will reach an equilibrium state, and so the bioaccessibility of PBDEs will not change. However, there was a dynamic accumulation of PBDEs in the Caco-2 cells because of the transport of PBDEs across the cells into the BL side

from the AP side. Because the addition of oil and starch was able to significantly improve the bioaccessibility, there were obvious differences between the bioaccessibility and accumulation ratios of PBDEs in the static in vitro method and the dynamic cell model. The protein and dietary fiber in the digestion solution might absorb released PBDEs to form precipitates after centrifugation. From the perspective of nutritional factors, there are few documents about comparing the two methods in terms of organic compounds, although there are studies on the metal element absorption, and it is found that protein or fructose can effectively increase the absorption of metals, such as Zn, Fe, and so on (Maares and Haase, 2020; Rodriguez-Ramiro et al., 2019). In the present study, in the in vitro method, there is a mechanical agitation process that simulates gastrointestinal movement, and the experimental digestion time was consistent with the actual gastrointestinal digestion cycle. However, the Caco-2 cell model lack of a mechanical food stirring process, it may rely on the compounds bound to albumin and continuously transported within the blood circulation. Therefore, although Caco-2 cells may continue to absorb PBDEs from the medium, this has to compete with the adsorption of PBDEs by protein or dietary fiber. Also, the addition of protein and dietary fiber increase the absorption of PBDEs to some extent. To better model actual conditions, a dynamic simulation to determine the bioaccessibility may be more suitable, and hence further studies are needed.

4. Conclusions

This study showed that the transport and accumulation of PBDEs in a Caco-2 cell monolayer model and bioaccessibility of PBDEs determined using an in vitro gastrointestinal digestion method highly depended on the type of nutrients, including oil, starch, protein and dietary fiber. Although the nutrients were all found to be excellent in facilitating the release of PBDEs in the gastrointestinal system, there were some differences between the in vitro method and cell model regarding the bioaccessibility and accumulation of PBDEs. The presented results have important implications for investigating the impact of nutrients on human exposure to PBDEs and associated health risks. However, due to the great complexity, there are some shortcomings in both methods for simulating the actual human gastrointestinal environment. Thus, further research is needed.

CRediT authorship contribution statement

Xiaojing Li: Methodology, Data analysis, Design, Draft preparation. **Mengmeng Wang**: Methodology, Experiment, Data analysis. **Yan Yang**: Data analysis, Draft preparation. **Bingli Lei**: Methodology. **Shengtao Ma**: Data analysis. **Yingxin Yu**: Design, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (41907299, 41977303, and 41991310) and the Innovation Team Project of Guangdong Provincial Department of Education (2017KCXTD012).

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