

Original Research

Fate Determination and Characterization of Food Additive Silicon Dioxide and Titanium Dioxide in Commercial Foods

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Abstract

Background: Silicon dioxide (SiO₂) and titanium dioxide (TiO₂) are ones of the most widely used food additives as an anti-caking and a coloring agent, respectively, in the food industry. Understanding particle, aggregate, or ionic fates of two additives in commercial products is of importance to predict their potential toxicity. **Methods:** Triton X-114 (TX-114)-based cloud point extraction (CPE) methods for two additives were optimized in food matrices. Their particle or ionic fates in various commercial foods were determined by the CPE, and the physico-chemical properties of separated particles were further characterized. **Results:** SiO₂ and TiO₂ were primarily present as particle forms without changes in constituent particle size, size distribution, and crystalline phase. The maximum solubilities of SiO₂ and TiO₂ were 5.5% and 0.9%, respectively, depending on food matrix type, supporting their major particle fates in complex food matrices. **Conclusions:** These findings will provide basic information about the fates and safety aspects of SiO₂ and TiO₂ additives in commercial processed foods.

Keywords: silicon dioxide; titanium dioxide; cloud point extract; fate; particle size; crystalline phase; solubility

1. Introduction

Silicon dioxide (SiO₂) and titanium dioxide (TiO₂) are the most widely used food additive particles and have been widely applied to the food industry as an anti-caking and a coloring agent, respectively [1–3]. SiO₂ is used in confectionary, powdered mixtures, and seasonings to prevent ingredients from clumping together. TiO₂ is mainly added in confectionary including chocolates, candies, bakeries, snacks, and chewing gums due to its whitening pigment property. In the United States, the Food and Drug Administration recommends that the amounts for SiO₂ and TiO₂ are below 2% and 1% by weight of the food, respectively [1,2]. In the European Union (EU), SiO₂ and TiO₂ were registered as E551 with maximum levels at 1% in dried powdered foods and as E171 with no maximum level specified, respectively [4,5]. Current regulations do not specify the particle size range or size distribution of food additive SiO₂ and TiO₂. Rapid development of nanotechnology may lead to manufacture nano-sized SiO₂ and TiO₂ particles, which have large specific surface area to volume ratio, high reactivity, and different biological responses compared with bulk-sized particles [6,7]. Indeed, recent studies demonstrate that nanoparticles (NPs) ranged from 1 to 100 nm are present in commercially available food additive SiO₂ and TiO₂, raising concerns about their potential toxicity [8–11].

Food additives are utilized in processed foods where thermal, mixing, and agitating treatments are often applied, which can cause the formation of aggregates or dissolution of particles [4,12]. Moreover, the interactions between food

additives and food components can occur, which can also affect the fates of food additive particles [13–16]. Determining whether SiO₂ and TiO₂ are present as intact particles, aggregates, or dissolved forms is important to understand and predict their potential toxicity. Orally taken SiO₂ is generally considered to be not toxic at actual usage levels [17–19]. However, some contradictory results were also reported, showing its potential toxicity in terms of oxidative stress, inflammation response, and intestinal barrier dysfunction, although most studies were performed using *in vitro* systems [20–22]. In the EU, food additive TiO₂ (E171) is considered no longer safe due to uncertainty of its genotoxicity [23]. TiO₂ is known to induce reactive oxygen species (ROS), which is critical to cause oxidative stress-related diseases [15,24]. Recently studies demonstrated that food-grade TiO₂ can be involved in the intestinal barrier dysfunction, colorectal cancer, and development in offspring rodents [22,25,26]. Hence, the information about the fates of SiO₂ and TiO₂ will be necessary and useful to answer the question as whether their toxicity is related to particle or ionic fates.

Most studies on the fate determination of NPs have applied harsh conditions such as acid and heat treatments to digest organic matrices, which can surely alter their dissolution and particle fates [15,27]. A detergent-based cloud point extraction (CPE) was used for the detection of ions in matrices and environments after filtering, acid treatment, and dry-ashing [28,29]. In the previous study, we developed a Triton X-114 (TX-114)-based CPE method to separate zinc oxide (ZnO) particles as intact forms from com-



mercial foods and biomatrices without pre-treatments [14]. The CPE approach was also developed for SiO₂ in biomatrices and its fates were determined in cell lines and tissues [17]. In this study, the CPE method was further optimized for the most widely applied food additive particles, SiO₂ and TiO₂, in food matrices to determine their dissolved, aggregated, or particle fates in commercial food products. Furthermore, the physicochemical properties of separated SiO₂ and TiO₂ particles from commercial foods by the CPE were characterized in terms of constituent particle size, crystalline phase, and solubility.

2. Materials and Methods

2.1 Materials

Food additives SiO₂ and TiO₂ particles were purchased from Evonik Industries AG (Essen, Germany) and Tioxide Europe S.R.L (Varese, Italy), respectively. TX-114, casein, humic acid (HA, sodium salt), Si standard solution, and Ti standard solution were provided by Sigma-Aldrich (St. Louis, MO, USA). Nitric acid (HNO₃), hydrogen peroxide (H₂O₂), hydrofluoric acid (HF), sodium chloride (NaCl), sodium hydroxide (NaOH), and ethyl alcohol were supplied by Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Gyeonggi-do, Republic of Korea). Powdered sugar was purchased from Samyang Co. (Seoul, Republic of Korea). Conical-bottom glass centrifuge tubes (15 mL) were obtained from Daeyoung Science (Seoul, Republic of Korea). Commercial products containing SiO₂ (candy, powder, coffee mix, milk tea, and snacks) or TiO₂ (candy, powder, sauces, chocolate, and snacks) as food additives were purchased from supermarkets located in Seoul, Republic of Korea in 2022.

2.2 Characterization

Particle size and shape of SiO₂ or TiO₂ were analyzed by field emission transmission electron microscope (FE-TEM; JEM-2100F, JEOL, Tokyo, Japan) equipped with energy-dispersive X-ray spectroscopy (EDS). The particles were dispersed in distilled and deionized water (DDW) and the suspensions (0.1 mg/mL) were prepared in ethyl alcohol solution and sonicated for 15 min (160 W, Branson 5800, Branson Ultrasonics, Danbury, CT, USA). The suspensions (5 μ L) were dropped on a carbon-coated copper grid (200 mesh, PELCO® TEM Grids, Ted Pella Inc., Redding, CA, USA). After drying at room temperature, TEM images were acquired at an accelerating voltage of 200 kV. The average particle sizes and size distributions of SiO₂ or TiO₂ particles were determined using ImageJ software (version 1.53k, National Institutes of Health, Bethesda, MD, USA).

Crystal structure of SiO₂ or TiO₂ was determined by powder X-ray diffraction (XRD) patterns using X-ray diffractometer (SmartLab, Rigaku Co., Tokyo, Japan) with Ni-filtered CuK α radiation (λ = 1.5418Å, a voltage of 40 kV, a current of 40 mA, a scan range of 5–80° with a step size of 0.02°, and a scanning rate of 3°/min).

Hydrodynamic diameters and zeta potentials of SiO₂ or TiO₂ were measured by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively, using a Zetasizer Nano System (Malvern Instruments, Worcestershire, UK). SiO₂ or TiO₂ suspension (0.1 mg/mL) was prepared in DDW or humic acid (HA) solution (10 μ g/mL) and stirred for 30 min. After further sonication for 15 min, the suspensions (1 mL) were immediately put in plastic cuvettes and the measurements were carried out at room temperature.

2.3 Optimizations of CPE for SiO₂ and TiO₂

CPE method for SiO₂ and TiO₂ was optimized with SiO₂ or TiO₂ dispersions (0.1 mg/mL) in DDW or HA solution (10 μ g/mL), respectively. After stirring for 30 min and sonication for 15 min at room temperature, the suspensions (7 mL) were transferred to bottom glass centrifuge tubes (15 mL) and the pH was adjusted to 3.0 and 4.0 for SiO₂ and TiO₂, respectively, with NaOH or HNO₃ solution. Next, TX-114 (0.5 mL of 5% (w/v)) and NaCl (0.75 mL of 0.2 M) solutions were added in the suspensions and diluted to 10 mL with DDW. The mixed solutions were incubated for 30 min at 45 °C to promote phase separation and centrifuged at 2500 \times g for 5 min at 25 °C. The precipitates and the supernatants where particles and ions were present, respectively, were digested for inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis (JY2000 Ultrace, HORIBA Jobin Yvon, Longjumeau, France) as described in “2.7. Digestion of Organic Materials, Microwave digestion, and ICP-AES Analysis”.

2.4 CPE Application for SiO₂ and TiO₂ in Food Matrices

The CPE developed for SiO₂ or TiO₂ was applied by spiking SiO₂ or TiO₂ into representative food matrices, powdered sugar or casein. The contents of SiO₂ and TiO₂ were adjusted to be 2% (w/w) and 1% (w/w) based on FDA recommendations, respectively. SiO₂ suspension (0.1 mg/mL) was spiked into 0.1 g of powdered sugar or casein at the concentration of 2% (w/w) and dispersed in DDW (7 mL). TiO₂ (0.1 mg/mL) was spiked into 0.1 g of food matrices at the concentration of 1% (w/w) and dispersed in HA solution (7 mL). After stirring for 30 min and sonication for 15 min, the same procedure was applied as described in “2.3. Optimizations of CPE for SiO₂ and TiO₂”.

2.5 Fate Determination of SiO₂ and TiO₂ in Commercial Foods

Commercial foods (10 g), such as candy, powder, coffee mix, milk tea, chocolate, sauce, and snacks, containing SiO₂ or TiO₂ as a food additive on product labeling were homogenized in an agate mortar. Homogenized commercial products were dispersed in 7 mL of DDW and 7 mL of HA solution for the determination of SiO₂ and TiO₂, respectively. After stirring for 30 min and sonication for 15 min, the same procedure was applied as described in “2.3.

Optimizations of CPE for SiO₂ and TiO₂".

2.6 Dissolution Properties of SiO₂ and TiO₂ in Food Matrices and Commercial Foods

Dissolution properties of SiO₂ and TiO₂ in food matrices were evaluated in powdered sugar or casein, spiked with SiO₂ (2% (w/w)) or TiO₂ (1% (w/w)). Commercial foods (10 g) containing SiO₂ or TiO₂ as a food additive indicated on product labeling were homogenized in an agate mortar. Food matrices (0.1 g) or homogenized commercial products (0.1 g) spiked with SiO₂ and TiO₂ were dispersed in 10 mL of DDW and HA, respectively, and stirred for 30 min, followed by sonication for 15 min at room temperature. Further incubation was carried out for 30 min at 45 °C, as described in "2.3. Optimizations of CPE for SiO₂ and TiO₂". The suspensions were then centrifuged at 16,000×g for 15 min to collect the supernatants containing dissolved Si or Ti. The Si and Ti concentrations in the supernatants were determined by ICP-AES analysis after pre-digestion with HNO₃ and H₂O₂ as described in "2.7. Digestion of Organic Materials, Microwave Digestion, and ICP-AES Analysis".

2.7 Digestion of Organic Materials, Microwave Digestion, and ICP-AES Analysis

Si or Ti concentrations were quantified by measuring total Si or Ti contents using ICP-AES analysis, with Si or Ti standard solutions of different concentrations based on our previous reports [15,30]. It was reported that the amount of SiO₂ and TiO₂ in commercial foods are ranged from 1.5 to 7 mg/g and 0.2 to 10 mg/g, which are enough to be detected by ICP-AES [15,31,32]. Organic materials in the supernatants obtained after dissolution or CPE experiments were digested with 10 mL of ultrapure HNO₃ and 1 mL of H₂O₂ at 180 °C until the solution was colorless and entirely evaporated. The precipitates containing SiO₂ or TiO₂ particles after CPE application were digested in perfluoroalkoxy microwave digestion vessels using a microwave system (ETHOS EASY, Milestone Srl, Sorisole, Italy). Briefly, SiO₂ particles were digested with 6 mL of 70% HNO₃ and 1 mL of 40% HF. TiO₂ particles were digested with 6 mL of 70% HNO₃ and 2 mL of 40% HF [33,34]. The samples were digested for 55 min at 1600 W by irradiation at 120, 160, and 210 °C for 15, 10, and 30 min, respectively, followed by holding for 1 min. After digestion, all samples were diluted to suitable volumes with DDW, shaken up and down, and analyzed by ICP-AES. Method blanks were determined by performing the same procedure in the absence of Si or Ti samples. Instrument operating conditions are described in **Supplementary Table 1**.

2.8 Statistical Analysis

Results were presented as means ± standard deviations. One-way analysis of variance with Tukey's test was performed using the SAS Ver.9.4 (SAS Institute Inc., Cary,

NC, USA) to determine the significances of intergroup differences. Statistical significance was accepted for *p* values of <0.05.

3. Results and Discussion

3.1 Optimization of CPE for SiO₂ and TiO₂

To optimize TX-114-based CPE methods for SiO₂ and TiO₂, commercially available food-grade SiO₂ and TiO₂ were purchased and the characterization of each particle was carried out. **Supplementary Fig. 1A,B** show that the constituent particle sizes of SiO₂ and TiO₂ by TEM analysis were 14 ± 4 nm and 109 ± 35 nm, respectively. Zeta potential values of SiO₂ and TiO₂ under different pH conditions are presented in **Supplementary Fig. 1C**, showing isoelectric points (IEP) of the former and the latter were pH 1.9 and 3.1, respectively. It was reported that the electrostatic repulsion of particles is minimized at pH close to the IEP due to reduction of Brownian motion, and thus, the pH where zeta potentials are close to zero is optimal for the formation of NPs captured in TX-114-based micelles [35,36]. But, the addition of NaCl during the CPE process elevated zeta potential values of NPs. Hence, the pH values for the CPEs were set at higher pHs than IEP values, 3.0 and 4.0 for SiO₂ and TiO₂, respectively. As a consequence, particles can be captured in TX-114-based micells as precipitates, whereas ionized forms are present in supernatants after phase separation using CPE followed by centrifugation.

Table 1 demonstrates the changes in hydrodynamic diameters and zeta potentials before and after CPE application. The hydrodynamic diameters of SiO₂ suspension in DDW were statistically same to those obtained by CPE, whereas SiO₂ in HA solution had increased hydrodynamic diameters after CPE application (Table 1). In case of TiO₂, no increase in hydrodynamic diameters after CPE was found when it was dispersed in HA solution. It is worth noting that particles captured in TX-114 micelle by CPE must have the same particle size distribution compared with pristine particles without aggregation or dissolution, which is critical for fate determination as intact forms. Hence, SiO₂ and TiO₂ were dispersed in DDW and HA solution, respectively, for CPE application. This result also suggests that the CPE process can capture SiO₂ and TiO₂ as intact forms without aggregate formation or degradation. On the other hand, Table 1 shows that the zeta potential values of both SiO₂ and TiO₂ changed to less negative charges after CPE, probably resulted from NaCl addition during the CPE process.

Total recoveries of SiO₂ and TiO₂ were about 92.8% and 97.5%, respectively, and only 0.6% and 0.1% of SiO₂ and TiO₂ were detected as Si and Ti ionic forms, respectively, after CPE application (Fig. 1A,B). Meanwhile, the solubility of pristine SiO₂ and TiO₂ was assessed under the CPE conditions, showing 0.5% and 0.2% solubilities for the former and the latter, respectively, without significant differences between solubility and ionic fates (Fig. 1B). These

Table 1. Hydrodynamic diameters and zeta potentials of pristine food additive SiO₂ and TiO₂ under different CPE conditions.

Dispersant type	Hydrodynamic diameters (nm)		Zeta potentials (mV)	
	Before CPE	After CPE	Before CPE	After CPE
SiO ₂ in DDW	229 ± 3 ^{A,a}	226 ± 18 ^{A,a}	−37 ± 1 ^{A,a}	−27 ± 4 ^{B,b}
SiO ₂ in HA	466 ± 66 ^{B,a}	708 ± 170 ^{B,a}	−42 ± 1 ^{B,a}	−23 ± 2 ^{A,b}
TiO ₂ in DDW	304 ± 4 ^{A,a}	838 ± 22 ^{B,b}	−23 ± 1 ^{A,a}	−11 ± 1 ^{A,b}
TiO ₂ in HA	337 ± 11 ^{A,a}	354 ± 7 ^{A,a}	−32 ± 2 ^{B,a}	−14 ± 1 ^{B,b}

Different upper-case letters (A,B) indicate significant differences between different CPE dispersion conditions ($p < 0.05$). Different lower-case letters (a,b) indicate significant differences between before and after CPE steps ($p < 0.05$). Abbreviation: CPE, cloud point extract; DDW, distilled and deionized water; HA, humic acid.

results clearly indicate that SiO₂ and TiO₂ particles can be obtained as intact particle forms by CPE approaches.

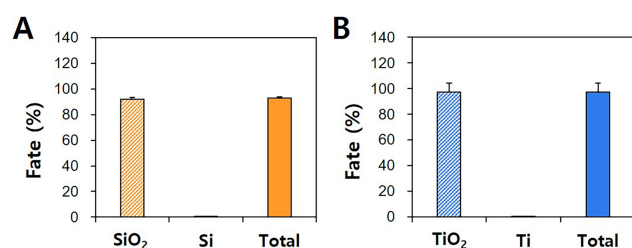


Fig. 1. Particle or ionic fates and total recoveries of pristine food additive (A) SiO₂ and (B) TiO₂ by CPE.

3.2 CPE Application for SiO₂ and TiO₂ in Food Matrices

Representative food matrices such as powdered sugar and casein were spiked with SiO₂ or TiO₂, and the CPE methods optimized were applied. Powdered sugar and casein were chosen as food matrices due to their frequent usage in confectionary. Fig. 2A shows that there were no statistical changes in hydrodynamic diameters between pristine SiO₂ or TiO₂ and particles recovered from particles-spiked powdered sugar or casein by CPE application, suggesting that the CPE methods can recover SiO₂ and TiO₂ particles from food matrices as intact forms without aggregation or dissolution. The recoveries of SiO₂ and TiO₂ in powdered sugar or casein as particle forms were ranged from 93.2% and 102.6% (Fig. 2B,C). Only ~0.7% and 0.1% of SiO₂ and TiO₂ were detected as ionic forms, respectively, regardless of matrix type. Total recoveries of both particles and ions were 93.3%–103.3% for all cases, implying reliability of the results. All the results suggest that SiO₂ and TiO₂ added in food matrices can be separated as intact particle forms by applying the CPE.

On the other hand, the solubility of SiO₂ and TiO₂ was also evaluated because particles can dissolve to some extent in food matrices. As shown in Fig. 2D, the solubilities of SiO₂ and TiO₂ were ~0.9% and 0.1%, respectively, without significant differences compared with ionic forms (%) obtained by CPE (Fig. 2B,C). This result clearly supports that

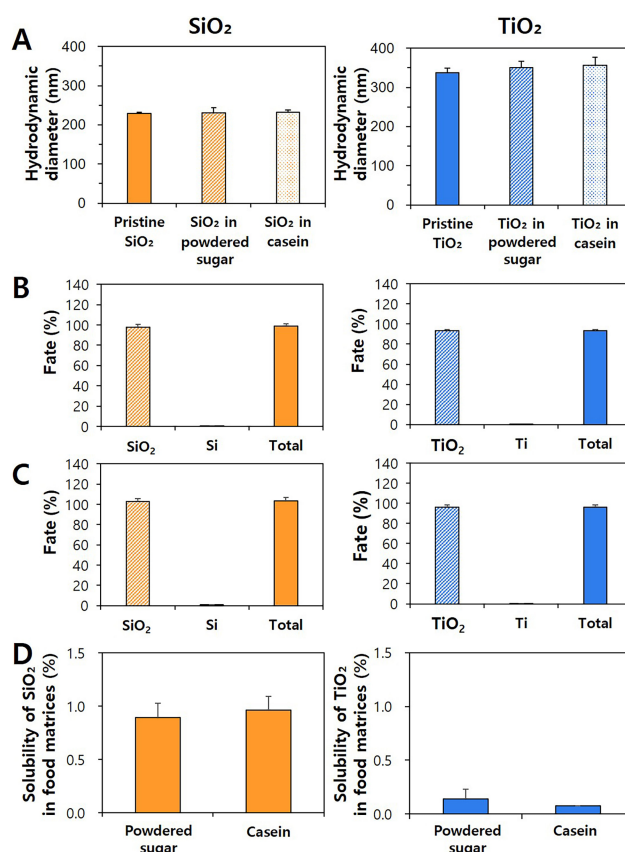


Fig. 2. Hydrodynamic diameters, fates, and solubility of SiO₂ and TiO₂ particles in food matrices. (A) Hydrodynamic diameters of SiO₂ and TiO₂ in food matrices before (pristine) and after CPE. No significant differences between before and after CPE were found ($p > 0.05$). Particle or ionic fates and total recoveries of pristine food additive SiO₂ and TiO₂ in (B) powdered sugar and (C) casein by CPE. (D) Dissolution properties of food additive SiO₂ and TiO₂ in food matrices.

the CPE methods can separate particles from ionic forms in food matrices.

Table 2. Recovery, coefficient of variation (CV), relative error (RE), limit of detection (LOD), and limit of quantification (LOQ) of quantitative analytical procedure for food additive SiO₂ and TiO₂.

Samples	Validation parameters	Concentrations ($\mu\text{g/mL}$)				
		0.5	1	5	10	
Pristine	SiO ₂	Recovery (%)	98.63 \pm 1.77	92.61 \pm 1.06	90.18 \pm 3.10	91.38 \pm 0.77
		CV (%)	1.80	1.14	3.43	0.84
		RE (%)	−1.37	−7.39	−9.82	−8.62
		LOD ($\mu\text{g/mL}$)			0.04	
		LOQ ($\mu\text{g/mL}$)			0.11	
	TiO ₂	Recovery (%)	96.26 \pm 1.47	93.53 \pm 1.33	96.58 \pm 0.38	95.38 \pm 1.02
		CV (%)	1.53	1.43	0.40	1.07
		RE (%)	−3.74	−6.47	−3.42	−4.62
		LOD ($\mu\text{g/mL}$)			0.01	
		LOQ ($\mu\text{g/mL}$)			0.03	
Powdered sugar	SiO ₂	Recovery (%)	107.54 \pm 8.33	105.94 \pm 10.27	108.32 \pm 2.66	101.16 \pm 5.90
		CV (%)	7.74	9.69	2.45	5.83
		RE (%)	7.54	5.94	8.32	1.16
		LOD ($\mu\text{g/mL}$)			0.10	
		LOQ ($\mu\text{g/mL}$)			0.30	
	TiO ₂	Recovery (%)	93.58 \pm 0.62	99.71 \pm 1.47	102.61 \pm 1.55	103.98 \pm 2.81
		CV (%)	0.67	1.48	1.51	2.70
		RE (%)	−6.42	−0.29	2.61	3.98
		LOD ($\mu\text{g/mL}$)			0.06	
		LOQ ($\mu\text{g/mL}$)			0.18	
Casein	SiO ₂	Recovery (%)	97.63 \pm 6.92	105.32 \pm 4.36	105.71 \pm 1.64	106.43 \pm 1.23
		CV (%)	7.09	4.14	1.56	1.16
		RE (%)	−2.37	5.32	5.71	6.43
		LOD ($\mu\text{g/mL}$)			0.03	
		LOQ ($\mu\text{g/mL}$)			0.10	
	TiO ₂	Recovery (%)	96.95 \pm 0.98	100.61 \pm 1.53	100.02 \pm 2.33	104.49 \pm 4.89
		CV (%)	1.01	1.52	2.33	4.68
		RE (%)	−3.05	0.61	0.02	4.49
		LOD ($\mu\text{g/mL}$)			0.13	
		LOQ ($\mu\text{g/mL}$)			0.38	

3.3 Validation of Analytical Methods for SiO₂ and TiO₂ in Food Matrices

Quantitative analytical methods using acid digestion in a microwave system were validated by calculating recovery (%), linearity (coefficient of determination values, R²), accuracy (relative error, RE), precision (coefficient of variation, CV), limit of detection (LOD), and limit of quantification (LOQ). Table 2 shows that the recoveries of pristine SiO₂ and TiO₂ or SiO₂- and TiO₂-spiked powdered sugar or casein were range from 90.18% to 108.32%. The CV and RE values for all the cases were 0.40%–9.69% and –9.82% to 8.32%, respectively. Fig. 3 demonstrates a good linearity for pristine particles and particles-spiked in food matrices. All the results indicate reliable analytical parameters for SiO₂ and TiO₂ in food matrices, which is consistent with other previous reports [15,30,37,38].

On the other hand, the LOD and LOQ values were 0.03–0.10 $\mu\text{g/mL}$ and 0.10–0.30 $\mu\text{g/mL}$ for SiO₂, and 0.01–0.13 $\mu\text{g/mL}$ and 0.03–0.38 $\mu\text{g/mL}$ for TiO₂, respectively. These are similar or lower values compared with

those obtained by other results, showing that the LOD and LOQ were 0.07 $\mu\text{g/g}$ and 0.20 $\mu\text{g/g}$ for SiO₂, and 0.03–0.34 $\mu\text{g/mL}$ and 0.09–1.04 $\mu\text{g/mL}$ for TiO₂, respectively [15,31]. Sensitively low LOQ and LOD values were obtained, suggesting that the analytical methods by acid digestion in a microwave system are effective and reliable.

3.4 Separation of SiO₂ and TiO₂ from Commercial Foods and Their Fate Determination

The CPE methods were applied to eight commercial products available on the market indicating SiO₂ or TiO₂ as a food additive on product labelling. Fig. 4 shows that 90.9%–101.8% of SiO₂ were present as particle forms, only 0.02%–7.3% ionized forms were detected. When total recovery percentage (91.3%–108.3%) of both SiO₂ particles and Si ions from products was considered, about 93.5%–99.9% of added SiO₂ were found as intact particles. In case of TiO₂, particle forms were detected in the range of 92.4% to 105.9%, whereas ionic forms were only found at 0.1%–0.9% (Fig. 5). Based on total recovery (93.3%–106.3%)

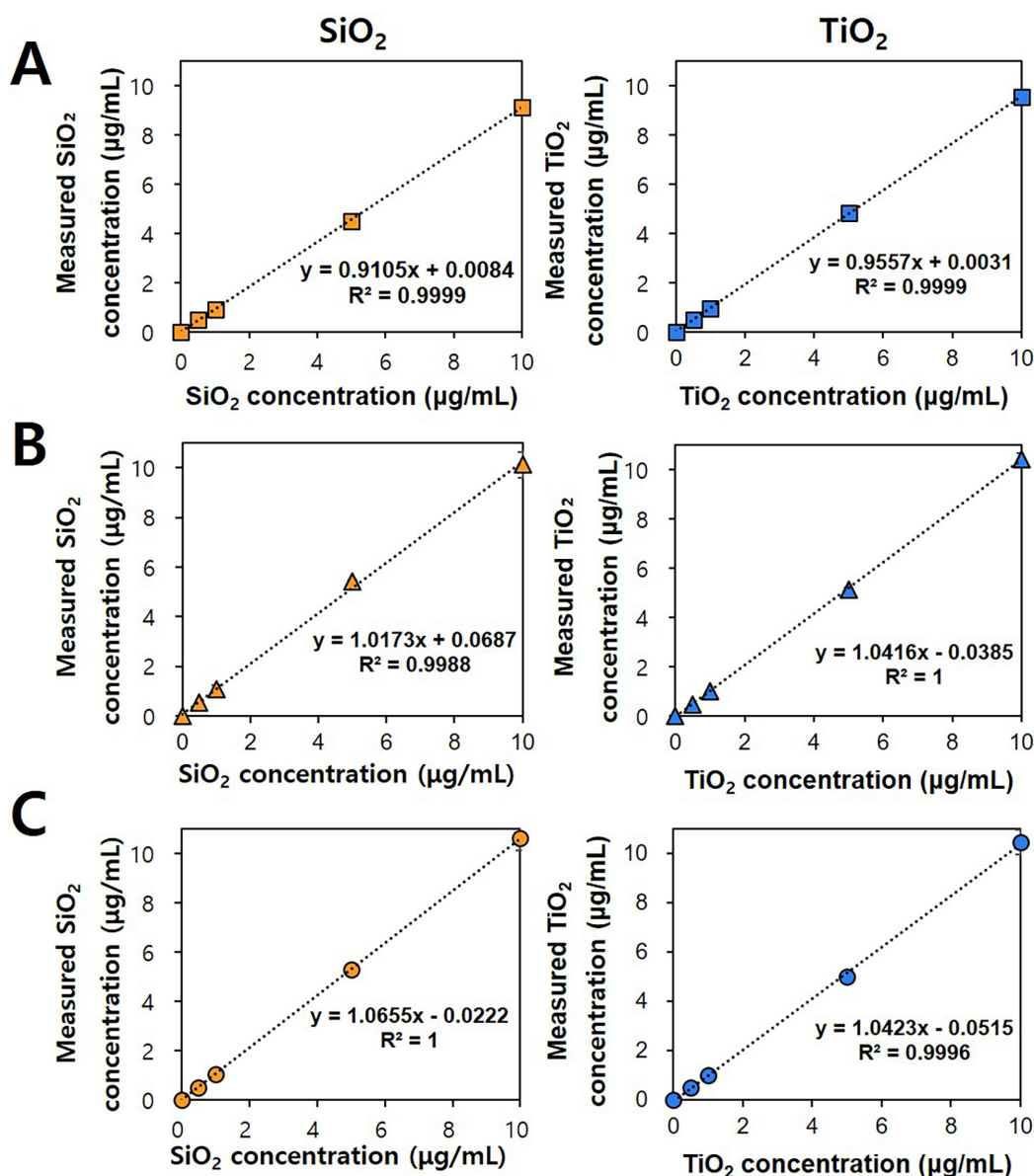


Fig. 3. Quantitative analysis of SiO₂ and TiO₂. Standard curves for (A) pristine food additive SiO₂ and TiO₂, (B) powdered sugar spiked with SiO₂ or TiO₂, and (C) casein spiked with SiO₂ or TiO₂ obtained by acid digestion in a microwave system, followed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis.

of both TiO₂ particles and Ti ions, about 99.1%–99.9% of added TiO₂ were detected as intact particle forms. The results demonstrate that most of food additive SiO₂ and TiO₂ are not degraded and present as particles, and these two additives have low solubility in food matrices. When detected ionic forms were compared, higher Si ions than Ti ions were found, probably indicating high solubility of the former than the latter. Indeed, it was reported that the solubility of SiO₂ was higher than TiO₂ [4,39,40], which is in good agreement with our results.

Dissolution property of SiO₂ and TiO₂ was further evaluated by spiking known amount of pristine SiO₂ and TiO₂ in commercial food matrices, the same products where the CPEs were applied (Figs. 4,5). As shown in Fig. 6A,B,

the solubilities of SiO₂ were ranged from 0.1% to 5.5%, whereas TiO₂ had 0.1%–0.9% solubilities depending on the matrix type. Overall results indicate that the solubility of two additives was low in commercial complex food matrices, but SiO₂ has high solubility than TiO₂, which is in good agreement with previous report [4,5]. It is worth noting that no significant differences between solubility (Fig. 6) and ionic fate (Figs. 4,5) were found in the same products ($p > 0.05$), indicating that the solubility critically affects the fates of two additives in foods. The results support reliable fate determination of SiO₂ and TiO₂ in commercial foods by CPE, and also suggest that major fates of SiO₂ and TiO₂ are particle forms in commercial processed products. Relatively high solubility of SiO₂ in powder 1, powder 2,

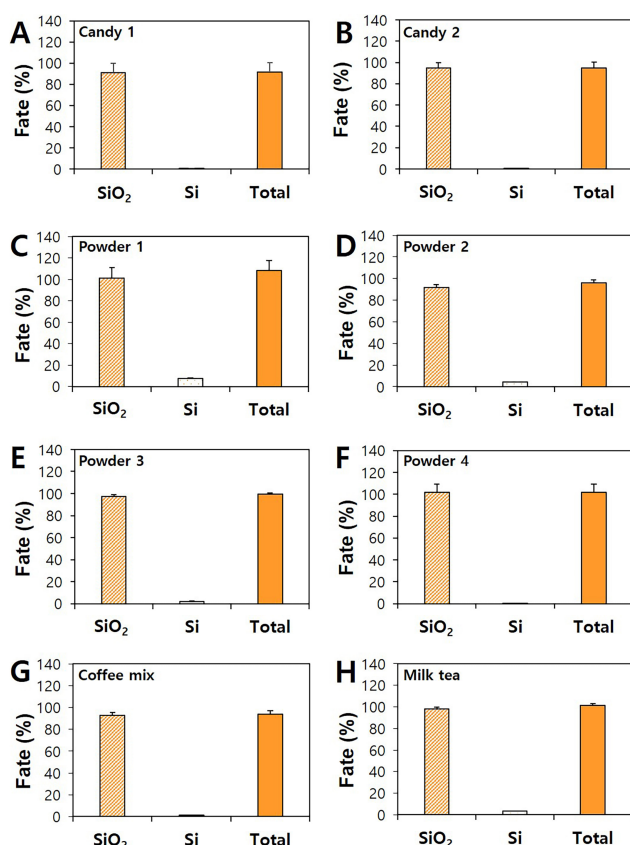


Fig. 4. Particle or ionic fates and total recoveries of SiO₂ in commercial foods by CPE. Particle or ionic fates and total recoveries of SiO₂ in commercial (A) candy 1, (B) candy 2, (C) powder 1, (D) powder 2, (E) powder 3, (F) powder 4, (G) coffee mix, and (H) milk tea by CPE.

and milk tea seems to be related to its matrix interactions because their pH values were neutral (pH ~7.0). Indeed, the interactions between food additive SiO₂ and food matrices were demonstrated, although the interactions seem to be not strong [3,6,13]. Quantitative analysis revealed that saccharides, proteins, fatty acids, and minerals can be adsorbed on the surface of SiO₂ [13]. Hence, the adsorption of food matrices on SiO₂ may increase its solubility in commercial food products. Meanwhile, the interaction between TiO₂ and food matrices is likely to be negligible based on its extremely low solubility (Fig. 6B) and minor ionic fates (Fig. 5).

3.5 Characterization of Separated SiO₂ and TiO₂ from Commercial Foods

Physico-chemical characterization of separated and recovered SiO₂ and TiO₂ from commercial food products by CPE was further carried out. The constituent particle sizes and size distributions were analyzed by TEM. Fig. 7A,B and Fig. 8A,B show that the constituent particle sizes of separated SiO₂ and TiO₂ were ranged from 12 ± 3 nm to 21 ± 4 nm and from 112 ± 30 nm to 132 ± 37 nm, respectively, without significant differences from pristine

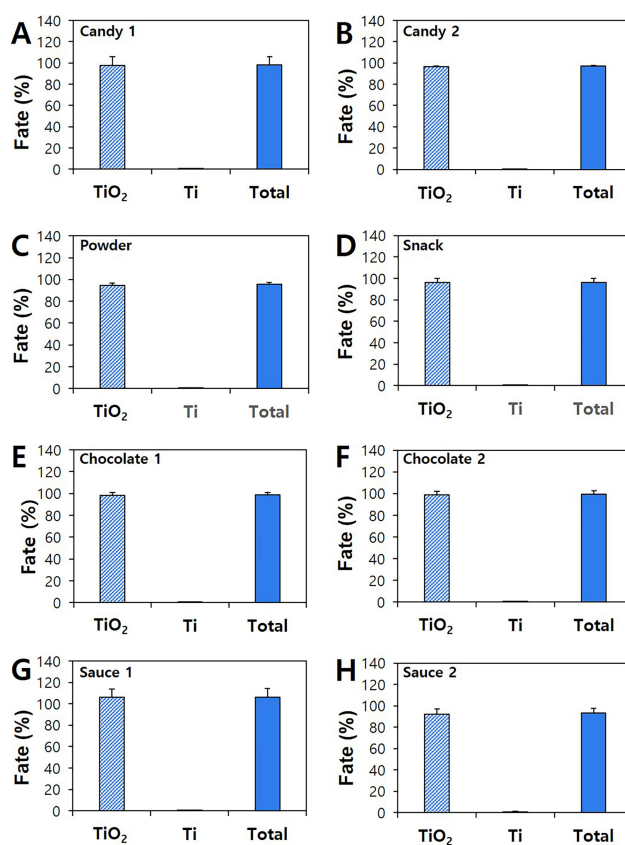


Fig. 5. Particle or ionic fates and total recoveries of TiO₂ in commercial foods by CPE. Particle or ionic fates and total recoveries of TiO₂ in commercial (A) candy 1, (B) candy 2, (C) powder, (D) snack, (E) chocolate 1, (F) chocolate 2, (G) sauce 1, and (H) sauce 2 by CPE.

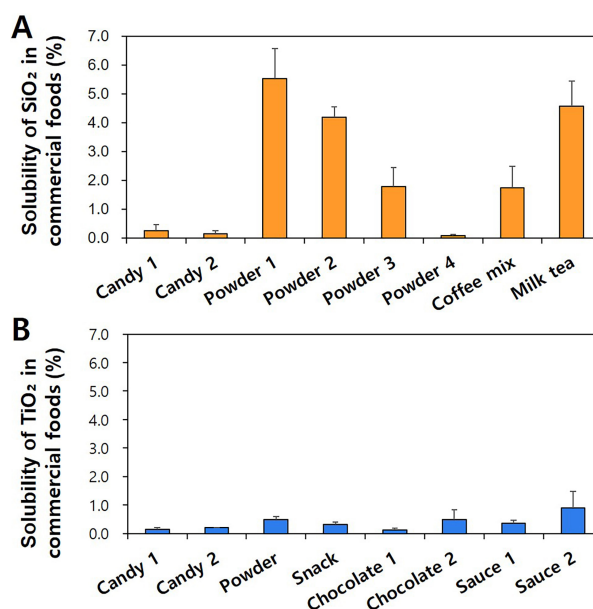


Fig. 6. Dissolution properties of (A) SiO₂ and (B) TiO₂ in commercial foods.

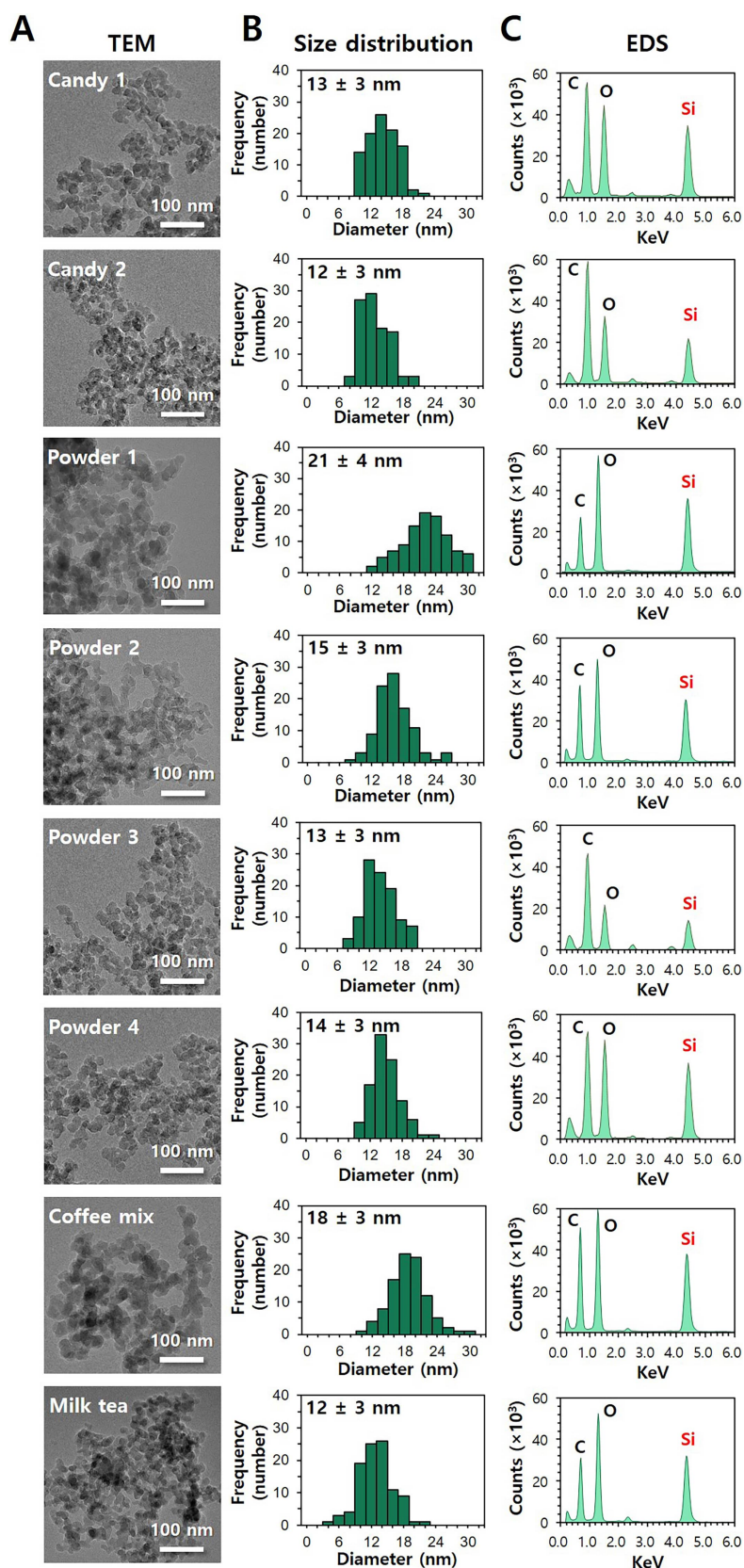


Fig. 7. Characterization of separated SiO₂ from commercial foods. (A) Transmission electron microscopy (TEM) images, (B) size distribution obtained by the TEM images, and (C) energy dispersive X-ray spectroscopy (EDS) of separated SiO₂ from commercial foods.

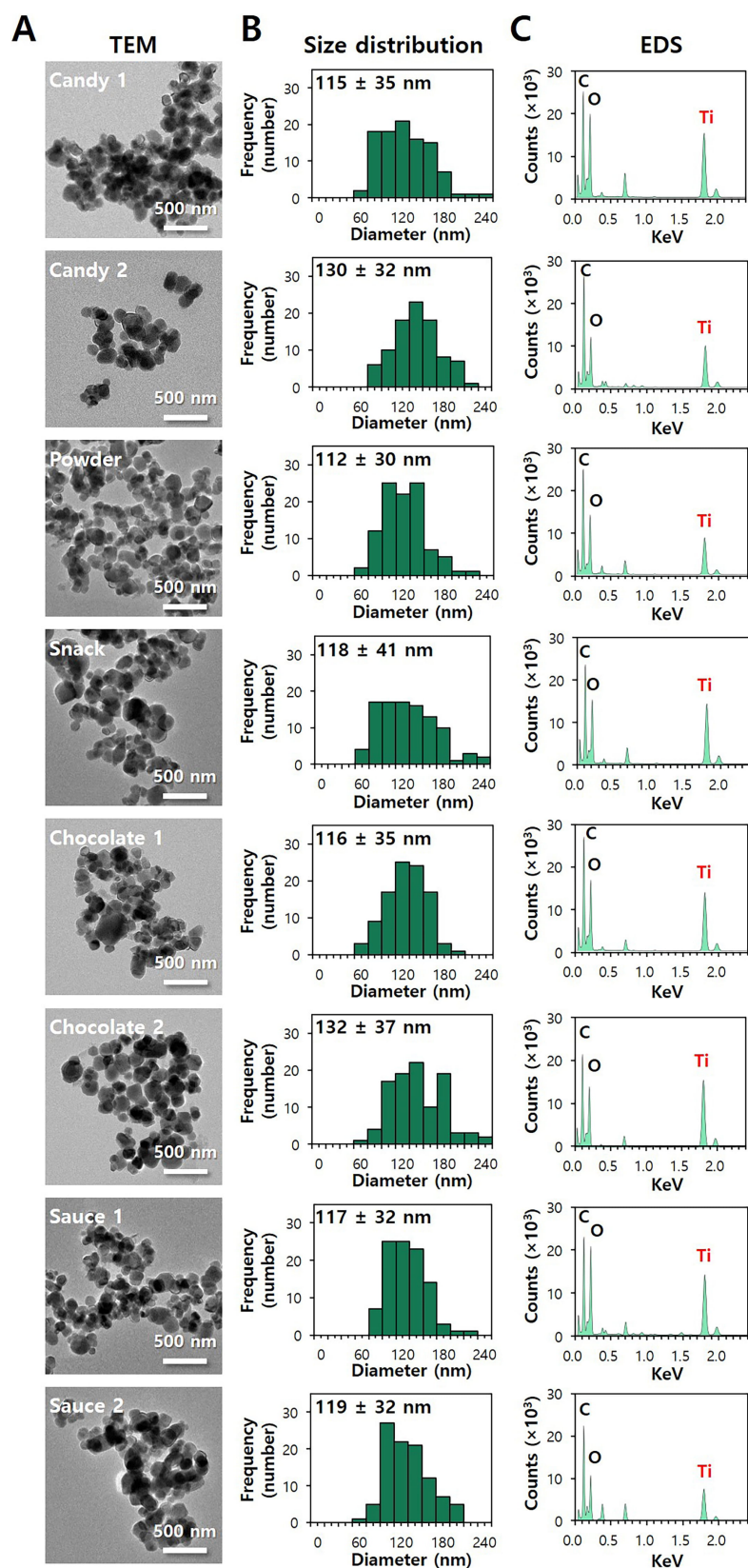


Fig. 8. Characterization of separated TiO_2 from commercial foods. (A) Transmission electron microscopy (TEM) images, (B) size distribution obtained by the TEM images, and (C) energy dispersive X-ray spectroscopy (EDS) of separated TiO_2 from commercial foods.

SiO₂ (14 ± 4 nm) and TiO₂ (109 ± 35 nm) ($p > 0.05$). No remarkable aggregate formation or decomposed form was observed. The TEM-EDS analysis clearly reveals the presence of Si or Ti ions in the particles observed (Figs. 7C,8C). The results confirm that food additive SiO₂ and TiO₂ are primarily present as particles without degradation or aggregation.

The crystalline phases of separated SiO₂ and TiO₂ from commercial foods were analyzed by XRD patterns. The results indicate that pristine SiO₂ and TiO₂ have typical amorphous and anatase phases, respectively, and the same crystalline phases were observed in separated SiO₂ and TiO₂ particles from commercial foods (Figs. 9,10). It should be noted that amorphous SiO₂ and anatase TiO₂ are authorized as food additives [3,23]. All the results imply that food additive SiO₂ and TiO₂ are present as intact particle forms without changes in crystalline phase and constituent particle size.

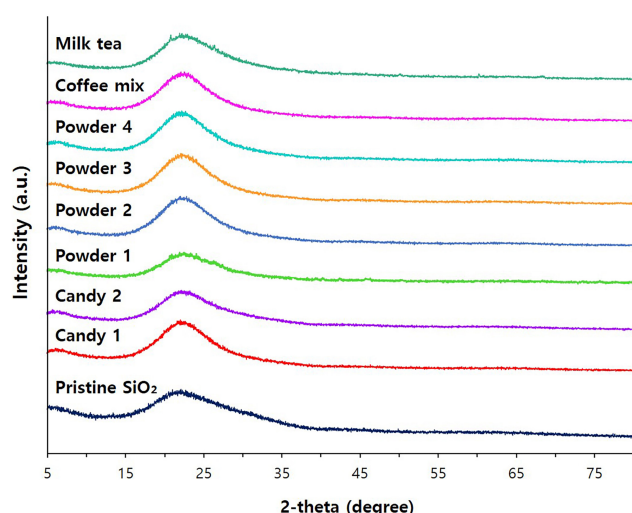


Fig. 9. X-ray diffraction (XRD) patterns of pristine food additive SiO₂ and separated SiO₂ from commercial foods.

4. Conclusions

Particle, aggregate, or ionic fates of food additive SiO₂ and TiO₂ in commercial processed foods were determined by optimizing CPE approaches in food matrices. The results reveal that most SiO₂ and TiO₂ in commercial foods were present as particle forms without significant differences in constituent particle size and size distribution compared with pristine food-grade each particle. Moreover, the crystalline phases of separated SiO₂ and TiO₂ from processed foods were not affected, showing amorphous and anatase crystalline phases for the former and the latter, respectively. The ionic fates of two additives by CPE were highly consistent with the solubility results, demonstrating maximum ~5.5% and ~0.9% ionized forms for SiO₂ and TiO₂, respectively. Higher ionic fate and dissolution of

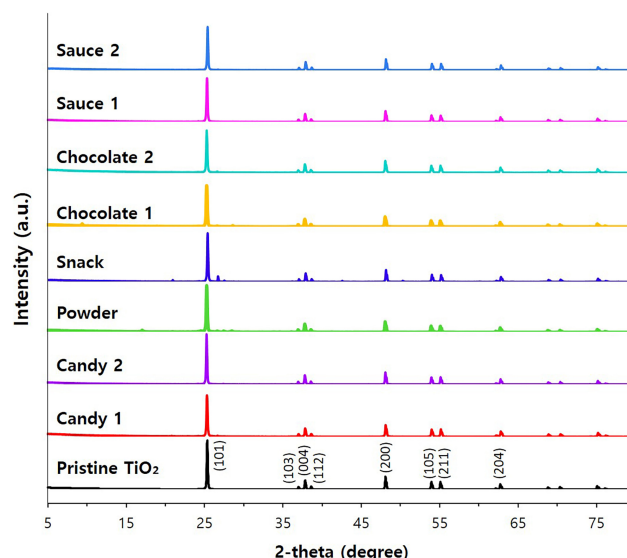


Fig. 10. X-ray diffraction (XRD) patterns of pristine TiO₂ particles and separated TiO₂ from commercial foods.

SiO₂ than TiO₂ in foods were found, indicating that the former can more easily dissolve in food matrices. The interactions between NPs and matrices may affect their dissolution fates in food products. All the results suggest that most food additive SiO₂ and TiO₂ were present as intact particle forms, implying that particle fates of food additives should be considered for understanding and predicting their potential toxicity.

Availability of Data and Materials

All data generated or analyzing during this study are included in this published article.

Author Contributions

R-YK and S-BK performed the research and analyzed the data. S-MY provides help and advice on experiments. S-JC designed the research study and wrote the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. S-JC is serving as one of the Guest editors of this Journal. We declare that S-JC had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GOC.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2802036>.

References

- [1] FDA. CFR - Code of Federal Regulations Title 21. 2022. Available at: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.480> (Accessed: 29 December 2022).
- [2] FDA. CFR - Code of Federal Regulations Title 21. 2022. Available at: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=73.575> (Accessed: 29 December 2022).
- [3] GSFA. General standard for food additives-codex stan 192-1995. 2021. Available at: <https://www.fao.org/fao-who-codex-alimentarius/codex-texts/dbs/gsf/en/> (Accessed: 29 December 2022).
- [4] Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipic M, Frutos MJ, *et al.* Re-evaluation of silicon dioxide (E 551) as a food additive. *EFSA Journal*. 2018; 16: e05088.
- [5] Aguilar F, Crebelli R, Domenico AD, Dusemund B, Frutos MJ, Galtier P, *et al.* Re-evaluation of titanium dioxide (E 171) as a food additive. *EFSA Journal*. 2016; 14: e04545.
- [6] Lee JA, Kim MK, Song JH, Jo MR, Yu J, Kim KM, *et al.* Biokinetics of food additive silica nanoparticles and their interactions with food components. *Colloids Surf B Biointerfaces*. 2017; 150: 384–392.
- [7] Guisbiers G, Mejía-Rosales S, Leonard Deepak F. Nanomaterial Properties: Size and Shape Dependencies. *Journal of Nanomaterials*. 2012; 2012: 1–2.
- [8] Medina-Reyes EI, Rodríguez-Ibarra C, Deciga-Alcaraz A, Diaz-Urbina D, Chirino YI, Pedraza-Chaverri J. Food additives containing nanoparticles induce gastrototoxicity, hepatotoxicity and alterations in animal behavior: The unknown role of oxidative stress. *Food and Chemical Toxicology*. 2020; 146: 111814.
- [9] Diao J, Xia Y, Jiang X, Qiu J, Cheng S, Su J, *et al.* Silicon dioxide nanoparticles induced neurobehavioral impairments by disrupting microbiota-gut-brain axis. *Journal of Nanobiotechnology*. 2021; 19: 174.
- [10] Pinget G, Tan J, Janac B, Kaakoush NO, Angelatos AS, O'Sullivan J, *et al.* Impact of the food additive titanium dioxide (E171) on gut microbiota-host interaction. *Frontiers in Nutrition*. 2019; 6: 57.
- [11] Bischoff NS, de Kok TM, Sijm D, van Breda SG, Briede JJ, Castenmiller JJM, *et al.* Possible adverse effects of food additive E171 (titanium dioxide) related to particle specific human toxicity, including the immune system. *International Journal of Molecular Sciences*. 2020; 22: 207.
- [12] Youn SM, Choi SJ. Food additive zinc oxide nanoparticles: dissolution, interaction, fate, cytotoxicity, and oral toxicity. *International Journal of Molecular Sciences*. 2022; 23: 6074.
- [13] Go MR, Bae SH, Kim HJ, Yu J, Choi SJ. Interactions between food additive silica nanoparticles and food matrices. *Frontiers in Microbiology*. 2017; 8: 1013.
- [14] Jeon YR, Yu J, Choi SJ. Fate determination of ZnO in commercial foods and human intestinal cells. *International Journal of Molecular Sciences*. 2020; 21: 433.
- [15] Hwang JS, Yu J, Kim HM, Oh JM, Choi SJ. Food additive titanium dioxide and its fate in commercial foods. *Nanomaterials*. 2019; 9: 1175.
- [16] Moradi M, Razavi R, Omer AK, Farhangfar A, McClements DJ. Interactions between nanoparticle-based food additives and other food ingredients: A review of current knowledge. *Trends in Food Science & Technology*. 2022; 120: 75–87.
- [17] Yoo NK, Youn SM, Choi SJ. Oral Toxicokinetics, Tissue Distribution, and 28-Day Oral Toxicity of Two Differently Manufactured Food Additive Silicon Dioxides. *International Journal of Molecular Sciences*. 2022; 23: 4023.
- [18] Liang CL, Xiang Q, Cui WM, Fang J, Sun NN, Zhang XP, *et al.* Subchronic oral toxicity of silica nanoparticles and silica microparticles in rats. *Biomedical and Environmental Sciences*. 2018; 31: 197–207.
- [19] Kim YR, Lee SY, Lee EJ, Park SH, Seong NW, Seo HS, *et al.* Toxicity of colloidal silica nanoparticles administered orally for 90 days in rats. *International Journal of Nanomedicine*. 2014; 9: 67–78.
- [20] Hanot-Roy M, Tubeuf E, Guilbert A, Bado-Nilles A, Vigneron P, Trouiller B, *et al.* Oxidative stress pathways involved in cytotoxicity and genotoxicity of titanium dioxide (TiO₂) nanoparticles on cells constitutive of alveolo-capillary barrier in vitro. *Toxicology in Vitro*. 2016; 33: 125–135.
- [21] Madhubala V, Pugazhendhi A, Thirunavukarasu K. Cytotoxic and immunomodulatory effects of the low concentration of titanium dioxide nanoparticles (TiO₂ NPs) on human cell lines - An in vitro study. *Process Biochemistry*. 2019; 86: 186–195.
- [22] Pedata P, Ricci G, Malorni L, Venezia A, Cammarota M, Volpe MG, *et al.* In vitro intestinal epithelium responses to titanium dioxide nanoparticles. *Food Research International*. 2019; 119: 634–642.
- [23] Younes M, Aquilina G, Castle L, Engel KH, Fowler P, Fernandez MJF, *et al.* Safety assessment of titanium dioxide (E171) as a food additive. *EFSA Journal*. 2021; 19: e06585.
- [24] Dorier M, Tisseyre C, Dusserre F, Beal D, Arnal ME, Douki T. Toxicological impact of acute exposure to E171 food additive and TiO₂ nanoparticles on a co-culture of Caco-2 and HT29-MTX intestinal cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2019; 845: 402980.
- [25] Urrutia-Ortega IM, Garduno-Balderas LG, Delgado-Buenrostro NL, Freyre-Fonseca V, Flores-Flores JO, Gonzalez-Robles A, *et al.* Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model. *Food and Chemical Toxicology*. 2016; 93: 20–31.
- [26] Hong F, Zhou Y, Ji J, Zhuang J, Sheng L, Wang L. Nano-TiO₂ Inhibits Development of the Central Nervous System and Its Mechanism in Offspring Mice. *Journal of Agricultural and Food Chemistry*. 2018; 66: 11767–11774.
- [27] Luo K, Park H, Adra HJ, Ryu J, Lee JH, Yu J, *et al.* Charge-switchable magnetic separation and characterization of food additive titanium dioxide nanoparticles from commercial food. *Journal of Hazard Materials*. 2020; 393: 122483.
- [28] Samaddar P, Sen K. Cloud point extraction: A sustainable method of elemental preconcentration and speciation. *Journal of Industrial and Engineering Chemistry*. 2014; 20: 1209–1219.
- [29] Mortada WI. Recent developments and applications of cloud point extraction: A critical review. *Microchemical Journal*. 2020; 157: 105055.
- [30] Yoo NK, Jeon YR, Choi SJ. Determination of Two Differently Manufactured Silicon Dioxide Nanoparticles by Cloud Point Extraction Approach in Intestinal Cells, Intestinal Barriers and Tissues. *International Journal of Molecular Sciences*. 2021; 22: 7035.
- [31] Ka MH, Lee KG, Lim HY, Lee G, Yun SS, Lim HS, *et al.* An-

- alytical Method of Silicon Dioxide in Health Functional Food Products using ICP-OES. *Journal of Food Hygiene and Safety*. 2017; 32: 343–347.
- [32] Sungur S, Kaya P, Koroglu M. Determination of titanium dioxide nanoparticles used in various foods. *Food Additives and Contaminants Part B Surveill*. 2020; 13: 260–267.
- [33] Peters R, Kramer E, Oomen AG, Rivera ZE, Oegema G, Tromp PC, *et al*. Presence of nano-sized silica during in vitro digestion of foods containing silica as a food additive. *ACS Nano*. 2012; 6: 2441–2451.
- [34] Peters R, Undas AK, Memelink J, van Bommel G, Munniks S, Bouwmeester H, *et al*. Development and validation of a method for the detection of titanium dioxide particles in human tissue with single particle ICP-MS. *Current Trends in Analytical and Bioanalytical Chemistry*. 2018; 2: 74–84.
- [35] Liu JF, Chao JB, Liu R, Tan ZQ, Yin YG, Wu Y, *et al*. Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters. *Analytical Chemistry*. 2009; 81: 6496–6502.
- [36] Majedi SM, Kelly BC, Lee HK. Evaluation of a cloud point extraction approach for the preconcentration and quantification of trace CuO nanoparticles in environmental waters. *Analytica Chimica Acta*. 2014; 814: 39–48.
- [37] Santos EJD, Santos MPD, Herrmann AB, Sturgeon RE. Rapid determination of Ti in TiO₂ by ICP OES. *Analytical Methods*. 2016; 8: 6463–6467.
- [38] Yu J, Kim YH, Kim HM, Oh JM, Kim YR, Choi SJ. Determination of the fate and biological responses of food additive silica particles in commercial foods. *Food Chemistry*. 2020; 331: 127304.
- [39] Lenher V, Merrill HB. The Solubility of Silica. *Journal of the American Chemical Society*. 1917; 39: 2630–2638.
- [40] Avramescu ML, Chénier M, Palaniyandi S, Rasmussen PE. Dissolution behavior of metal oxide nanomaterials in cell culture medium versus distilled water. *Journal of Nanoparticle Research*. 2020; 22: 222.