Determination and identification of titanium dioxide nanoparticles in confectionery foods using inductively coupled plasma optical emission spectrometry and transmission electron microscopy

Namhoon Kim[a*], Changkyu Kim[b], Soyoung Jung[a], Youngju Lee[a], Juyeon Jo[a], Misun Hong[a], Sangmi Lee[a], Younghee Oh[a] and Kweon Jung

[a]*Food & Drug Division, and* [b]*Atmospheric Research Division, Seoul Metropolitan Government Research Institute of Public Health and Environment, Gyeonggi-do, Korea*

*Corresponding author.

Tel : +82-2-570-3223; Fax : +82-2-964-8174, E-mail : nhkim70@seoul.go.kr

Co-authors.

Changkyu Kim[b] : Tel : +82-2-570-3172, E-mail : ckkim0707@seoul.go.kr

Soyoung Jung[a] : Tel : +82-2-570-3226, E-mail : syjung12@seoul.go.kr

Youngju Lee[a] : Tel : +82-2-570-3227, E-mail : neo798@seoul.go.kr

Juyeon Jo[a] : Tel : +82-2-570-3228, E-mail : jyjo12@seoul.go.kr

Misun Hong[a] : Tel : +82-2-968-5097, E-mail : mshong7@seoul.go.kr

Sangmi Lee[a] : Tel : +82-2-570-3225, E-mail : 702lsm@seoul.go.kr

Younghee Oh[a] : Tel : +82-2-570-3221, E-mail : young9442@seoul.go.kr

Kweon Jung : Tel : +82-2-570-3303, E-mail : jungk719@seoul.go.kr

Abstract

Food-grade titanium dioxide (TiO_2) is a common and widespread food additive in many processed foods, personal care products, and other industrial categories as it boosts the brightness and whiteness of colors. Although it is generally recognized as safe for humans, there is a growing interest in the health risks associated with its oral intake. This study quantified and identified TiO_2 nanoparticles present in confectionery foods, which are children's favorite foods, with inductively coupled plasma optical emission spectrometry (ICP-OES) and transmission electron microscopy (TEM). A reliable digestion method using hot sulfuric acid and a digestion catalyst (K_2SO_4:$CuSO_4$ = 9:1) was suggested for titanium analysis. Validations of the experimental method were quite acceptable in terms of linearity, recoveries, detection limits, and quantification limits. Of all the 88 analyzed foods, TiO_2 was detected in 19 products, and all of them except for three declared TiO_2 in their labeling. The mean TiO_2 content of candies, chewing gums, and chocolates were 0.36 mg g^{-1}, 0.04 mg g^{-1}, and 0.81 mg g^{-1}, respectively. Whitish particles isolated from the confectionery foods were confirmed as TiO_2 nanoparticles via TEM and energy dispersive X-ray spectroscopy (EDX), in which nanosized particles (< 100 nm) were identified.

Keywords: titanium dioxide, confectionery foods, nanoparticles, ICP-OES, TEM

Introduction

Food-grade TiO_2 is widely applied as a food additive for brightening and whitening the color of confectionery foods, such as chocolates, candies, and chewing gums (Mutsuga et al. 2011). In addition, TiO_2 is commonly used as a white pigment in paints and personal care products including toothpastes and sunscreens (Weir et al. 2012). The industrial application of TiO_2 in various foods, particularly in nonwhite foods, such as dried vegetables, soups, cheeses, sauces, and dietary supplements, is reported to enhance the whiteness and opacity of these commodities, and it also helps them resist discoloration (Lim et al. 2015; Peters et al. 2014). The current annual production of TiO_2 is estimated at approximately 5.1 Mt throughout the world, and it is anticipated to continue increasing the use of TiO_2, which has primarily been produced as nanosized particles for many years (Landsiedel et al. 2010). It is generally known that the diameter of TiO_2 particles is 50-300 nm, and the majority of commercial products that use TiO_2 as a pigment additive are consisted with this particle size (Johnson et al. 1997). For special purpose applications such as photocatalysis, ultrafine TiO_2 particles of size of 1-150 nm are commonly utilized in cosmetics and sunscreens (Braun 1997). Meanwhile, a recent study demonstrated that the proportion of food-grade nanoscale TiO_2 (E171) particles that were < 100 nm in diameter corresponds to 36% as the application of coloring agents (Weir et al. 2012). The development of nanotechnology has led to the exploitation of various nanomaterials that are suitable for use in the food industry to improve the taste and texture of foods, and those applications in the food sector are promising. It is generally known that nano-engineered particles have unique physicochemical and biological properties compared to their original materials. These transformed properties (e.g., increased surface area and chemical reactivity) mean that the nanoscale materials may provoke unexpected toxicological effects on human health and the environment (Dekkers et al. 2011).

To date, numerous studies have reported that exposure to nanoscale materials through adsorption or inhalation can induce potential health risks in human cells and the environment, resulting in decreased apoptosis, increased oxidative stress, interrupted immune function and DNA damage, as nanoparticles can easily penetrate targeted human tissues and cells (Athinarayanan et al. 2015; Chang et al. 2015; Chen et al. 2014; Smolkova et al. 2015; Warheit et al. 2015).

TiO_2 is generally approved as a safe food additive for coloring agents by many food organizations including the US FDA, EFSA and the Korean FDA. The US FDA permits manufacturers to use up to 1% food-grade TiO_2 without declaring it on ingredient labels (PHYS ORG. 2015). According to the report of Weir et al. (2012), the oral exposure of US children under 10 years old to TiO_2 was 1–2 mg Ti/kg body weight per day. The carcinogenic properties of TiO_2 classify it as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC), which means that TiO_2 could possibly act as a carcinogen in humans (IARC. 2010).

Many studies have reported the measurement of TiO_2 in different matrices using a UV/VIS spectrometer, ICP-OES, ICP-MS, and single particle ICP-MS (Boguhn et al. 2009; Bussel et al. 2010; Khosravi et al. 2012; Krystek et al. 2014; Laborda et al. 2013; Lomer et al. 2000; Myers et al. 2004; Sharif et al. 2015; Vidmar et al. 2017; Weir et al. 2012). Prior to this instrumental analysis, all methods must be processed with a wet-ash digestion step using various mineral acids such as nitric acid (HNO_3), hydrochloric acid (HCl), hydrofluoric acid (HF), and sulfuric acid (H_2SO_4). Generally, the combination of HNO_3 and HF has been used for the dissolution of the TiO_2 contained in samples in various experiments (Krystek et al. 2014; Peters et al. 2014; Weir et al. 2012). Although HF has a superior ability to dissolve glass and inorganic oxides, it is unsuitable if considering the health of researchers because its chemical properties are very toxic, corrosive, and hard to handle. In addition, when using HF as a digestion reagent, special apparatus is required to analyze TiO_2, which means that HF is incompatible with general instruments equipped with glass components of ICP-OES and ICP-MS.

In this study we adopted a previous method (Lomer et al. 2000) to measure TiO_2 using ICP-OES with hot sulfuric acid as a digestion reagent and made the method more efficient at dissolving TiO_2 with a digestion catalyst, which is a combination of potassium sulfate and copper (Ⅱ) sulfate. Therefore, the aims of this study were to develop a reliable and efficient method for the determination of TiO_2 with ICP-OES in confectionery foods that are largely consumed by children and to characterize its morphological properties using TEM. The technique of electron microscopy is currently understood as a common method for characterizing and measuring engineered nanoparticles in food and consumer products (Calzolai et al. 2012; Dudkiewicz et al. 2015).

Materials and Methods

Samples

All confectionery foods were obtained from retail markets located in Seoul, Korea. The samples could be categorized into four groups, which were candies (n = 43), chewing gums (n = 16), chocolates (n = 20), and snacks (n = 9). Attempts were made to collect at least ten samples for each product group. Certain products that had a label bearing TiO_2 as an ingredient were preferentially selected for the experimental samples, but some collected samples did not have TiO_2 listed as an ingredient. All samples were ground homogeneously using a laboratory blender (Robot-Coupe, Vincennes, France) and kept refrigerated at -20℃ until use.

Chemicals and materials

Titanium (Ti) standard for ICP-OES analysis and the anatase crystalline form of TiO_2 reference material (99.8%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The rutile crystalline form of synthetic TiO_2 reference material (99.0%) and hydrogen peroxide (H_2O_2) (30.0%) were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). All chemicals and reagents used were analytical grade. Sulfuric acid (H_2SO_4) (96.0–98.0%) and nitric acid (HNO_3) (60.0–62.0%) were supplied from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Potassium sulfate (K_2SO_4) (98.0%) and Copper (Ⅱ) sulfate ($CuSO_4$) (98.0%) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ethanol for TEM pretreatment was supplied from Fisher Scientific Korea Ltd. (Seoul, Korea) and distilled water was purified using the Milli-Q integral 5 system (Millipore Co., Billerica, MA, USA).

Sample digestion

Each sample of 0.5 g was accurately weighed in a borosilicate glass tube (Kjeldahl tube). After that, 10 mL of concentrated sulfuric acid and 1 g of digestion catalyst, which was composed of potassium sulfate and copper (Ⅱ) sulfate (9:1), were added to the tube. The tube containing the sample was digested at 400 ℃ for two hours using a Kjeldahl digest unit (Digest Automat K-438 connected with Scrubber B-414) from Buchi Labortechnik AG (Flawil, Switzerland). After cooling at ambient temperature, the digested solution, which appeared as transparent and yellowish blue because of the added digestion catalyst, was transferred into a 100 ml volumetric flask that was then filled up with distilled water. Finally, the diluted solution with about 10% sulfuric acid was used as a test solution.

ICP-OES measurement

The Ti content in the test solutions was measured using a PerkinElmer Optima 8300 ICP-OES (Waltham, MA, USA) equipped with an autosampler (Elemental Scientific, Inc., Omaha, NE, USA) and a Peek Mira Mist nebulizer. The ICP-OES operating conditions were as follows: 1.5 ml min^{-1} sample flow rate, 45 psi nebulizer pressure, 200 ml min^{-1} auxiliary gas flow rate, 12000 ml min^{-1} plasma gas flow rate, 550 ml min^{-1} carrier gas flow rate, and 1500 W RF power. Ti was determined at the wavelength of 336.12 nm.

Transmission Electron microscopy

Samples for TEM imaging were prepared by dropping 5–10 µl of the solution containing TiO_2 particles dispersed in ethanol on to 200-mesh carbon-coated copper grids obtained from Agar Scientific (Stansted, UK), and the pretreated samples were disposed at room temperature until they reached solid dryness. The morphology and size of isolated particles were investigated using a TECNAI G2 Sprit TEM (FEI, Czech Republic) with a 120 kV accelerating voltage. The analysis of the elemental composition of the nanosized TiO_2 structures was observed via energy dispersive X-ray spectroscopy (EDX) (EDX-720, Shimadzu, Kyoto, Japan).

Isolation of TiO_2 nanoparticles

Intact TiO_2 nanoparticles were isolated from the food commodities to observe TEM images by placing approximately 100 mg of ground samples in a 15 ml conical glass tube. A 3 ml digestion solution made by mixing 10 ml of hydrogen peroxide with 0.5 ml of nitric acid was poured into the tube, which was then heated in a dry block bath (MG-2200, Tokyo Rikakikai Co., Ltd., Japan) at 120 ℃ for 2–3 hours until the final volume nearly reached 2 ml. After cooling at room temperature, the remaining sample was transferred into a 2 ml centrifuge tube and ultrasonicated for 1 min. The samples were centrifuged at 10,000*g* for 20 min using a microcentrifuge (A32010(1), Gyrozen co. Ltd. Korea) to obtain whitish particles. The precipitated particles were collected and resuspended in 2 ml ethanol. This purification process was repeated twice to acquire pure particles that were used for the TEM experiment.

Calibration curve and recovery study

The Ti standard for ICP analysis was diluted with 10% sulfuric acid to make a stock solution at 100 mg kg^{-1} concentration. A calibration curve was prepared with 0.1, 0.2, 0.5, 1, 2, 5 and 10 mg kg^{-1} concentrations, obtained by serially diluting the stock solution. Samples of size 500 mg for three products including a candy, a chewing gum, and a chocolate that had been confirmed not to contain TiO_2 were fortified with approximately 10 mg and 50 mg TiO_2 reference material in rutile crystalline form to perform a recovery study. The spiked samples were then digested via the previously described method. After digestion, the recovery samples were treated using identical procedures for ICP analysis, and recovery experiments were performed in triplicate .

Results and discussion

Preliminary method validation

Linearity, limit of detection (LOD), limit of quantification (LOQ), and recoveries were measured to validate the described method analyzing TiO_2 additives contained in the confectionery food including candies, chewing gums, and chocolates using ICP-OES. Figure 1 shows a calibration curve for titanium ($^{47.9}Ti$) diluted in 10% sulfuric acid having linearity within the range of 0.1–10 mg kg^{-1} (r^2=0.999). It was reported that the concentration of Ti in the samples digested in 32.5% sulfuric acid was linear below 5 mg kg^{-1} due to the effect of acid suppression on emission intensity (Lomer et al. 2000). A recent study also proposed that the use of sulfuric acid as a digestion reagent was not suitable for the measurement of trace Ti concentration with ICP-MS owing to the interference mechanism between sulfur oxide species (S–O) and the primary Ti isotope, which all have the same *m/z* =48 (Weir et al. 2012). The final concentration of sulfuric acid was approximately adjusted to 10% to overcome these drawbacks, this meant that 10 ml of 96–98% sulfuric acid was diluted with 90 ml of distilled water in a 100 ml volumetric flask, and it was confirmed that the linearity of the calibration curve was acquired with up to 10 mg kg-1 using ICP-OES. In addition, experimental safety was secured in our present study because hydrofluoric acid, which can be both extremely harmful to humans and can damage analytical instruments, was not used as digestion reagent.

LOD and LOQ were measured by the signal-to-noise method, which utilizes the standard deviation (σ) of responses based on the slope (s) of the calibration curve. LOD and LOQ were calculated using $3.3 \times \sigma/s$ and $10 \times \sigma/s$, respectively. The LOD and LOQ values for Ti were respectively determined as 2.7 and 8.1 μg kg^{-1}, and the value means that our instrumental conditions had a lower detection limit for Ti analysis in comparison with the previous report, which showed 5.5 μg kg^{-1} in 32.5% sulfuric acid (Lomer et al. 2000). Another study (Peters et al. 2014) also reported that the quantification limit of digestion and detection method was observed in 0.01 mg Ti per g of product with ICP-MS. Actually, the value seemed to be considerably high, but no data in our experiment was below the value, which demonstrates that TiO_2 is added in the process of food manufacturing; therefore, it is expected that the minimum dose of addition is greater than 0.01 mg Ti per g of product.

The mean recoveries of TiO_2 from the control samples, which were confirmed as having no TiO_2 in preliminary tests, are as shown in Table 1. The recovery test was performed with three different matrices (a candy, a chewing gum, and a chocolate) in two different concentrations of added TiO_2. The calculated concentrations of Ti were acquired by multiplying the amount of added TiO_2 with the ratio of the molecular weight between Ti and TiO_2 (47.9/79.9 ≒ 0.59). When the average amount of added TiO_2 was in the range of 5.16 – 5.22 mg, the recoveries of TiO_2 were measured as the range of 91.58 ± 2.56–92.56 ± 0.35%, so it was confirmed that there was no significant difference among the matrices. A chocolate product that was fortified with 13.0 mg of TiO_2 showed the highest recovery rate of 98.65 ± 2.22%. In the case of a spiked chewing gum with 11.93 mg TiO_2, the recovery rate of TiO_2 was comparatively low at 93.90 ± 0.86%. These results are similar to those of a previous study (Lomer et al. 2000) that demonstrated that the mean recovery of TiO_2 suspended in gelatin was 95 ± 9.2% using ICP-OES. Another study (Peters et al. 2014) reported an average recovery of 96 ± 6% with ICP-MS.

***TiO_2* content in confectionery foods**

All 88 confectionery foods were collected from retail markets and were tested to measure the TiO_2 contents. Some foods listed TiO_2 as an ingredient, but some others did not include it on the label. In the case of foods that did not include TiO_2, tested samples that contained a hard coating shell or white color were primarily selected. Of all 88 products, TiO_2 was identified in 19. The analyzed results of the confectionery foods containing TiO_2 that were measured in triplicate are as shown in Table 2. The highest Ti concentrations of candies, chewing gums, and chocolates were 739.6 ± 15.3 mg kg^{-1}, 45.5 ± 6.6 mg kg^{-1}, and 1265.0 ± 18.4 mg kg^{-1}, respectively. Meanwhile, all snack foods were confirmed as having no TiO_2. Earlier studies (Peters et al. 2014; Weir et al. 2012) reported that chewing gum products had the highest concentration of Ti out of all surveyed products. In contrast, our results showed that the chewing gum products had lower Ti concentrations compared to other product categories. The reason is attributed to the difference among surveyed chewing gum products. In our study, the chewing gum products were primarily selected as children's favorite products that were usually sold in the nearby school zone, and its major appearance characteristics were various mixed colors and soft coating shells.

As shown in Table 2, the range of TiO_2 content per serving size was 0.06–105.5 mg g^{-1}, and the values greatly differed among different food items. Generally, as chocolate products have a comparatively larger mean serving size of approximately 36.5 g, the TiO_2 content per serving size also appeared to be larger than that of other products. According to the report of Churg (1996), when the added TiO_2 concentrations of various foods were calculated according to the intake dose, it was addressed that the daily intake of TiO_2 for an individual may exceed 200 mg. This proposal is fairly conceivable, considering our measured TiO_2 contents were limited to confectionery foods. Actually, in our daily lives, it is more likely that we will consume more various foods that contain TiO_2. In particular, children have a tendency to be commonly exposed to confectionery foods that contain TiO_2, and these are mostly consumed by children. In the report of Weir et al. (2012), which detailed human exposure to TiO_2 among the US population, children under 10 years old showed a 2–10 times higher exposure to TiO_2 per kg_{bw} than other consumer age groups.

Most TiO_2 detected products well obeyed the regulation of labeling TiO_2 as an ingredient, but some products (1 chewing gum and 2 chocolates) did not meet this labeling regulation. Although it is not possible for consumers to realize the exact quantities of TiO_2 contained in the foods they eat through studying their food's label information, they should be able to easily identify whether purchased foods contain TiO_2 by just browsing the foods label. Therefore, it is necessary that all food manufactures provide more accurate labeling information for the TiO_2 ingredient, and it can be helpful if consumers select foods that do not contain TiO_2. With the perspective of countries that manufacture products in which TiO_2 is detected, it was confirmed that TiO_2 is widely used all around world.

The distribution of TiO_2 content for food products can be compared using the normalized box plot for TiO_2 content shown in Figure 2. The average TiO_2 content of chocolate products was measured as 0.814 mg g^{-1} product, followed by candy products with 0.355 mg g^{-1} product, and chewing gum products with 0.042 mg g^{-1} product. In chocolate products, the difference between maximum and minimum values for TiO_2 content was very large. Meanwhile, in the case of chewing gum products, the difference among products was relatively small. In candy products, one product was observed as a statistical outlier because the TiO_2 content of it was significantly different from the other eight products. For three products (CH-2, CG-2, and CA-6) that had a hard outer coating shell, Ti concentrations were compared between the outer shell and inner portion of the products. As shown in Figure 3, it was confirmed that nearly 90% of Ti existed in the products' outer shell. These results were comparable to the Ti contents of gum products that had an outer shell as reported by Weir et al. (2012). Generally, chocolate products with a hard outer coating shell (CH-1, CH-2, CH-3, and CH-5), that was mainly made from tar colors, sugars, and TiO_2, showed the highest Ti concentration for all chocolate products. This means that the role of TiO_2 in foods is to provide color brightness and texture stabilization. Meanwhile, there were relatively few candy products with an outer shell (CA-5 and CA-6) out of all nine detected products. Soft candy products that did not have an hard outer shell such as jellies (CA-7 and CA-9) also had higher TiO_2 concentrations. In this case, TiO_2 is used to increase the transparency of products.

Identification of TiO_2 nanoparticles with TEM

Microscopic images of TiO_2 particles isolated from the confectionery foods were acquired via TEM to identify the particles' morphologies and sizes. As shown in Figure 4, it was confirmed that the morphological properties of TEM images were almost similar between two different TiO_2 reference materials (rutile and anatase) and the TiO_2 particles isolated from samples. All TEM images showed that the shape of particles were irregular and rounded with agglomerate properties, simultaneously coexisting with various particle sizes of 50–300 nm., It has been generally reported that approximately 10% of the TiO_2 particles contained in food products were < 100 nm, which was reconfirmed in our study. Despite the aggregation properties of TiO_2 particles in preparation for TEM analysis, the shape and size of particles possibly could be identified. In Figure 5, the analysis of EDX showed the presence of the Ti element, which has the largest peak in all analyzed peaks. The Cu peaks were considered to have originated from the copper grids utilized for TEM analysis. With the results of TEM and EDX, it was confirmed that the particles isolated from three products (CA-7, CG-2, and CH-2) were certainly TiO_2 nanoparticles.

Conclusion

The contents and morphological properties of TiO_2 nanoparticles present in confectionery foods were investigated using ICP-OES and TEM. The order of TiO_2 concentrations per serving of food products was chocolates, candies, and then chewing gums. In the case of foods that had an outer coating shell, more than 90% of TiO_2 existed in that outer shell. It could be confirmed that chocolate products with an outer shell are the largest contributors to TiO_2 intake among confectionery foods. The majority of products that included TiO_2 on the label as a food additive contained TiO_2 nanoparticles. In light of the recent situation in which many studies have reported potential health risks associated with the oral consumption of

TiO_2, it is required that children restrict their intake of confectionery foods that contain TiO_2 to lessen their risks of chronic exposure.

References

Athinarayanan J, Alshatwi AA, Periasamy VS, Al-Warthan AA. 2015. Identification of nanoscale ingredients in commercial food products and their induction of mitochondrially mediated cytotoxic effects on human mesenchymal stem cells. J Food Sci. 80:459-464.

Boguhn J, Baumgartel T, Dieckmann A, Rodehutscord M. 2009. Determination of titanium dioxide supplements in different matrices using two methods involving photometer and inductively coupled plasma optical emission spectrometer measurements. Arch Anim Nutr. 63:337-342.

Braun JH. 1997. Titanium dioxide : A review. J. Coatings Technol. 69:59-72.

Bussel WV, Kerkhof F, Kessel TV, Lamers H, Nous D, Verdonk H, Verhoeven B. 2010. Accurate determination of titanium as titanium dioxide for limited sample size digestibility studies of feed and food matrices by inductively coupled plasma optical emission spectrometry with real-time simultaneous internal standardization. Atom Spectroscopy. 31:81-88.

Calzolai L, Gilliland D, Rossi F, 2012. Measuring nanoparticles size distribution in food and consumer products: a review. Food Addit Contam Part A. 29:1183-1193.

Chang X, Xie Y, Wu J, Tang M, Wang B. 2015. Toxicological characteristics of titanium dioxide nanoparticles in rats. J Nanosci Nanotechnol. 15:1135-1142.

Chen T, Yan J, Li Y. 2014. Genotoxicity of titanium dioxide nanoparticles. J Food Drug Anal. 22:95-104.

Churg A. 1996. The uptake of mineral particles by pulmonary epithelial cells. Am J Respir Crit Care Med. 154:1124-1140.

Dekkers S, Krystek P, Peters RJB, Lankveld DPK, Bokkers BGH, Hoeven-Arentzen PHV, Bouwmeester H, Oomen AG. 2011. Presence and risks of nanaosilica in food products. Nanotoxicology. 5:393-405.

Dudkiewicz A, Boxall ABA, Chaudhry Q, Molhave K, Tiede K, Hofmann P, Linsinger TPJ. 2015. Uncertainties of size measurements in electron microscopy characterization of nanomaterials in foods. Food Chem. 176:472-479.

IARC. 2010. Carbon black, titanium dioxide and talc. Available from: http://monographs. iarc.fr/ENG/Monographs/vol93/mono93.pdf. Accessed 30 December 2017.

Johnson RW, Thiele ES, French RH. 1997. Light-scattering efficiency of white pigments: an analysis of model core-shell pigments vs. optimized rutile TiO_2. TAPPI J. 80:233-239.

Khosravi K, Hoque ME, Dimock B, Hintelmann H, Metcalfe CD. 2012. A novel approach for determining total titanium from titanium dioxide nanoparticles suspended in water and biosolids by digestion with ammonium persulfate. Anal Chim Acta 713:86-91.

Krystek P. Tentschert J, Nia Y, Trouiller B, Noel L, Goetz ME, Papin A, Luch A, Guerin T, de Jong WH. 2014. Method development and inter-laboratory comparison about the determination of titanium from titanium dioxide nanoparticles in tissues by inductively coupled plasma mass spectrometry. Anal Bioanal Chem. 406:3853-3861.

Laborda F, Bolea E, Jimenez-Lamana J. 2013. Single particle inductively coupled plasma mass spectrometry: A powerful tool for nanoanalysis. Anal Chem. 86:2270-2278.

Landsiedel R, Ma-Hock L, Kroll A, Hahn D, Schnekenburger J, Wiench K, Wohlleben W. 2010. Testing metal-oxide nanomaterials for human safety. Adv Mater. 22:1-27.

Lim JH, Sisco P, Mudalige TK, Sanchez-Pomales G, Howard PC, Linder SW. 2015. Detection and characterization of SiO_2 and TiO_2 nanostructures in dietary supplements. J Agric Food Chem. 63: 3144-3152.

Lomer MCE, Thompson RPH, Commisso J,Keen CL, Powell JJ. 2000. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. Analyst 125:2339-2343.

Mutsuga M, Sato K, Hirahara Y, Kawamura Y. 2011. Analytical methods for SiO_2 and other inorganic oxides in titanium dioxide or certain silicates for food additive specifications. Food Addit Contam. 28:423-427.

Myers WD, Ludden PA, Nayigihugu V, Hess BW. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci. 82:179-183.

Peters RJB, Bermmel GV, Herrera-Rivera Z, Helsper HPFG, Marvin HJP, Weigel S, Tromp PC, Oomen AG, Rietveld AG, Bouwmeester H. 2014. Characterization of titanium dioxide nanoparticles in food products: Analytical methods to define nanoparticles. J Agric Food Chem. 62: 6285-6293.

PHYS ORG. 2015. Dunkin' donuts ditches titanium dioxide - but is it actually harmful?. Available from: https://phys.org/news/2015-03-dunkin-donuts-ditches-titanium-dioxide.html. Accessed 30 December 2017.

Sharif HA, Rasha AAE, Ramia ZAB. 2015. Titanium dioxide content in foodstuffs from the Jordanian market: Spectrophotometric evaluation of TiO_2 nanoparticles. IFRJ. 22:1024-1029.

Smolkova B, Yamami NE, Collins AR, Gutleb AC, Dusinska M. 2015. Nanoparticles in food. Epigenetic changes induced by nanomaterials and possible impact on health. Food Chem Toxicol. 77:64-73.

Vidmar J, Milacic R, Scancar J. 2017. Sizing and simultaneous quantification of nanoscale titanium dioxide and a dissolved titanium form by single particle inductively coupled plasma mass spectrometry. Microchem J. 132:391-400.

Warheit DB, Brown SC, Donner EM. 2015. Acute and subchronic oral toxicity studies in rats with nanoscale and pigment grade titanium dioxide particles. Food Chem Toxicol. 84:208-224.

Weir A, Westerhoff P, Fabricius L, Hristovski K, Goetz NV. 2012. Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol. 46: 2242-2250.