International Food Research Journal 22(3): 1024-1029 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Titanium dioxide content in foodstuffs from the Jordanian market: Spectrophotometric evaluation of TiO_2 nanoparticles

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Article history

Received: 20 May 2014
Received in revised form: 27 November 2014
Accepted: 3 December 2014

Keywords

Foodstuffs
Titanium dioxide
TiO_2 nanoparticles
Myers' procedure UV Spectrophotometer
Wet digestion
Scanning electron microscope (SEM)

Abstract

Titanium dioxide (TiO_2) is recognized for its safe use as an additive (E171) in food and drugs. However, TiO_2 was the subject of recent studies regarding its toxicity and carcinogenicity. The new studies targeted TiO_2 nanoparticles that may exist in the pigment grade in small amounts. These recent investigations initiated a local survey in the Jordanian market to determine the TiO_2 content in the different types of foodstuffs to ensure that limits did not exceed the 1% by weight (as established by the Food and Drug Administration). Myers' spectrophotometric procedure for faecal samples was optimized and validated for TiO_2 analysis. In this study, TiO_2 was evaluated in 25 traditional foodstuffs in the Middle East (Tahini, Halawa, Jameed, Humus, gums and juice powders). Food grade TiO_2 supplied in the local market for the food industry was tested for TiO_2 nanoparticles by SEM. The data obtained in this study indicated the absence of TiO_2 from Tahini, Halawa, canned humus, and Jameed (for all tested brands); in accordance with the information provided in the labels. For the tested gums and juice powders, TiO_2 content also did not exceed 1%.

Introduction

Titanium dioxide (TiO_2) is a white solid in the form of anatase or rutile (Olmedo *et al*., 2008), in which commercially available TiO_2 white pigment is produced by sulfate- or chloride-based processes (Kuzensof, 2006). Pigment grade TiO_2 contains particle sizes ranging from 200 to 350 nm with a small fraction of TiO_2 colorless nanoparticles (< 100 nm) being also present. Food pigment grade TiO_2 with the lowest content of heavy metals has been used for long time for opacity and whiteness of food and drugs (Kuzensof, 2006); and as a digestion marker (Bussel *et al*., 2010). TiO_2 nanoparticles are approved by the FDA and European Food Safety Authority (EFSA) (Kuempel and Ruder, 2006).TiO_2 (E171) is approved by the FDA (Kuzensof, 2006) as a food additive to a level of up to 1% by weight, and up to 358 mg per dosage in drug tablets (Kuzensof, 2006). Furthermore, it is listed in the EU Annex II of regulation 1333/2008 as a permitted color additive in foods at Good Manufacturing Practices (GMP) levels (Kuzensof, 2006). Recently, the safety, toxicity, and carcinogenicity of TiO_2 to humans (Baan *et al*., 2006; Kuempel and Ruder, 2006; Warheit *et al*., 2007; Ramanakumar *et al*., 2008) as a food additive has been discussed (Kuzensof, 2006). Recently, a group of researchers measured the TiO_2 nanoparticles in E171 food grade, and they determined that 36%, of these particles form stable colloids in water, and that 1-5% of particles passed through a 0.45 μm filter (Weir *et al*., 2012). These nanoparticles have been considered as possible human carcinogens (Kuempel and Ruder, 2006).

The aim of this study was to scan the most consumed foodstuffs in the Jordanian market for TiO_2 content by a an optimized and validated method (Myers *et al*., 2004) to evaluate that the manufactures were not exceeding the 1% limit (Kuzensof, 2006). Atomic absorption spectroscopy was not used since it gave low signal under 10 ppm Ti in solution, whereas GFAAS had a drawback of memory effect that demanded the regular usage of modifiers. SEM was used to test the E171 in the local market for the approximate percentage of TiO_2 nanoparticles. The 25 investigated items were juice powders, gums, liquid Jameed (fermented salted dietary product), Halawa (a flake of confection of crushed sesame seeds in a base syrup), Tahini (Vegan sesame seed paste), and canned Hummus (a dip of mashed check peas with Tahini) from several manufacturers. Samples were treated according to Myers' optimized procedure (Myers *et al*.,2004), and then absorbance was measured spectrophotometrically at 410 nm .

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Materials and Methods

Instrumentation

All analyses were carried out using a UV Spectrophotometer system (Cary 100 Varian, Australia) at 410 nm with a 1cm quartz cuvette. Microbalance MXA5 (RADWAG-Poland) was used for weighing purposes. Digestion process was carried out on Cimarec digital hot plate from Thermo Scientific. Scanning Electron Microscope Inspect F50 was used to screen for TiO_2 nanoparticles.

Chemicals and reagents

All chemicals used were analytical grade. Concentrated sulfuric acid (98% assay from SDFCL fine-clean limited), hydrogen peroxide (30% (w/w) from Scharlu), Copper sulfate $CuSO_4$ and Potassium sulfate K_2SO_4 (99%) were supplied by JHD fine chemicals, China. Titanium 1000 mg/L certified stock standard solution $(NH_4)_2TiF_6$ (Merck), titanium dioxide (assay> 99% from Sigma Aldrich), gelatin (Sigma Aldrich), titanium dioxide food grade (Shanghai Nanling Chemical products). All the 25 foodstuffs were purchased from local stores and supermarkets in Amman, Jordan.

Myers' optimized digestion procedure

Duplicate accurate maximum weight close to 0.5000 g sample in 100 ml Pyrex beakers were digested with, 13 mL of 18 M H_2SO_4, 3.5 g K_2SO_4, and 0.40 g $CuSO_4$ at 310^0C on a hot plate for one hour and a half, then adding 4.0 ml concentrated HNO_3, and 4.0 ml concentrated HCl gradually to the hot solution for another half an hour, where beakers were covered by watch-glasses (Myers., et al used Kjeldahl digestion without HCl or HNO_3). Solutions were left to cool down for 30 minutes, where samples had blue to blue green color (catalyst color), indicating the complete digestion, followed by adding 10 mL of 30% H_2O_2. Finally, volume is brought up to 100 mL with deionized water in 100 ml volumetric flasks. Samples were measured spectrophotometrically at 410 nm in a 1cm quartz cuvette.

Titanium (IV) calibration solutions

Based on a spectrophotometric procedure (Myers *et al*.,2004), two calibration curves for low and high concentrations of Ti(IV) were prepared. Solutions of 0, 1, 2, 4, 6, 8, 10, and 12 mg/L for low concentrations, and 0, 20, 40, and 60 mg/L for high concentrations were prepared by spiking the appropriate volumes of the stock 1000 mg/L solution into 100 ml beakers, following the steps in Myers' optimized digestion procedure. The corresponding TiO_2 concentrations were calculated from the corresponding molecular weight ratio of TiO_2 to Ti. Similar calibration curves were also established from directly weighing 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 10 mg of TiO_2 for low concentrations and 0, 2, 4, and 6 mg of TiO_2 for high concentrations, this was done to confirm reproducibility of the calibration curves using either solid TiO_2 standards or Ti aqueous stock solutions from different source.

Preparation of Foodstuffs samples solutions

Twenty five food samples were treated according to Myers' optimized procedure in duplicates.

Percent recovery experiment

Samples of titanium dioxide (0.20 – 6.0 mg) were suspended in 15% gelatin (organic material that is devoid from TiO^2) solution at 50°C which was then cooled to room temperature to form solid gelatin blocks (Lomer *et al*., 2000), then the resulted solid blocks were digested and analyzed as mentioned above. TiO_2 concentration results are compared with the same TiO_2 concentrations in non-gelatin matrix.

Standard addition method

Standard addition method was applied to one of the samples in order to evaluate the matrix effect and accuracy of external calibration curves (Thompson and Ellison, 2005). Shampart powder juice with peach favor was chosen. The sample was homogenized into fine powder using a grinder, where five portions of 0.08 g of powder juice in Pyrex beakers were spiked with 0, 2, 4, 6, and 8 mg/L, respectively as Ti from the 1000 mg/L stock solution (corresponding TiO_2 concentrations were calculated), then digested, and analyzed as above. Another 0.15 g portion of the Shampart powder juice was digested and analyzed for its TiO_2 content according to the prescribed procedure above using the external calibration curves.

Scanning Electron Microscope

TiO_2 (E171) food grade supplied from the local market in Jordan was scanned for the average particle size and for the presence of nanoparticles which are defined as < 100 nm in one dimension (Shi *et al*., 2013).

Results and Discussion

TiO_2 calibration curves

Two linear TiO_2 calibration curves were established for low and high range concentrations. The range was chosen based on the most gathered foodstuffs items which indicated products free

Table 1. TiO_2 content in foodstuffs

Food stuff item	Brand/ Country	Portion size/g	TiO_2 mg/ portion	% TiO_2/DI**	manufacturers' labels
Jameed	Bent- El- Basha/ Jordan	A verge serving for 1 persons/ 160 g	< LOD*	---------	E 171 is not included in the ingredients' label
Jameed	Karak/ Jordnan	A verge serving for 1 persons/ 160 g	< LOD	---------	E 171 is not included in the ingredients' label
Jameed	Jdoodna/ Jordan	A verge serving for 1 persons/ 160 g	< LOD	---------	E 171 is not included in the ingredients' label
Jammed	Jameedna- Zaman/ Jordan	A verge serving for 1 persons/ 160 g	< LOD	---------	E 171 is not included in the ingredients' label
Halawa with pistachios	Al-Nakhala/ Jordan	Average slice / 34 g	< LOD	---------	E 171 is not included in the ingredients' label
Halawa with pistachios	Dura/ Jordan	Average slice / 34 g	< LOD	---------	E 171 is not mentioned in the ingredients label
Halawa extra with pistachios	Aiam Zaman/ Jordan	Average slice / 34 g	< LOD	---------	E 171 is not included in the ingredients' label
Halawa	Ayesh/ Jordan	Average slice / 34 g	< LOD	---------	Free from food additives
Tahina	Kasih gold /Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Tahina	Star premium/ Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Tahina	El- asha/Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Tahina	Dura/ Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Tahina	Argan/ Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Tahina	Swelem/ Jordan	Average tablespoon/5.00 g	< LOD	----------	E 171 is not included in the ingredients' label
Canned humus	Beroni/ Jordan	Average tablespoon/1.50 g	< LOD	----------	E 171 is not included in the ingredients' label
Powder juice Peach flavor	Shampart/Turkey	Pack /10 g	83.20 mg	0.83 % 0.05	Ingredients label indicated the addition of TiO_2 (E 171)
Powder juice Lemon	Shampart/Turkey	Pack /10 g	80.29 mg	0.80 % 0.05	Ingredients label indicated the addition of TiO_2 (E 171)
Powder juice	Tang/ Egypt	Pack /25 g	57.12 mg	0.23 % 0.01	Ingredients label indicated the addition of TiO_2 (E 171)
Powder juice	Squeez/ Syria	Pack /45 g	21.70 mg	0.48 % 0.003	Ingredients label indicated the addition of TiO_2 (E 171)
Chewing-Gum	Fusen bubble/ Japan	Piece/4.2312 g	< LOD	----------	E 171 is not included in the ingredients' label
Chewing-Gum	Sharawi/ Jordan	Piece/ 1.5100 g	0.24 mg	0.017 % 0.003	Ingredients label indicated the addition of TiO_2 (E 171)
Chewing-Gum	Tictac/ Ireland	Piece/ 0.5000 g	0.44 mg	0.09 % 0.005	Ingredients label indicated the addition of TiO_2 (E 171)
Chewing-Gum	Extra/ Poland	Piece/ 1.4200 g	2.74 mg	0.19 % 0.05	Ingredients label indicated the addition of TiO_2 (E 171)
Chewing Gum with mint flavor	Mentos/ Vietnam	Piece/ 1.5200 g	8.92 mg	0.59 % 0.14	Ingredients label indicated the addition of TiO_2 (E 171)
Chewing Gum with green tea flavor	Mentos/ Vietnam	Piece/ 1.9400 g	17.69 mg	0.91 % 0.22	Ingredients label indicated the addition of TiO_2 (E 171)

*LOD: Limit of detection.

** DI: Daily intake in mg TiO_2 nanoparticles per body weight of 60 Kg

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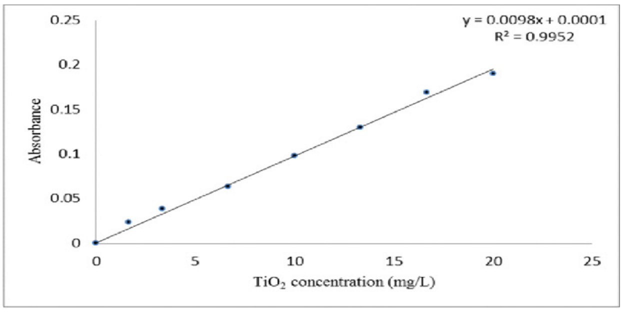
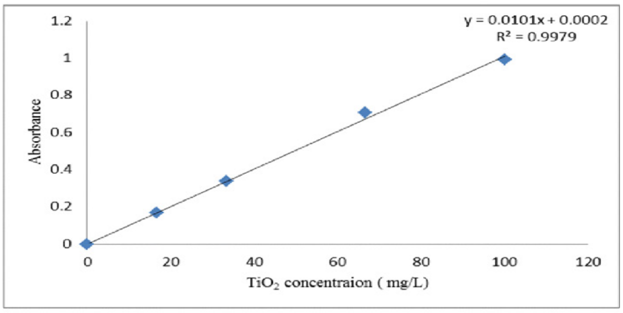
from TiO_2 or those with expected small amounts (low concentrations‘ calibration curve), while other products indicated the addition of considerable amounts of TiO_2 (calibration curve for higher concentrations). The obtained calibration equation was y = 0.0098x+ 0.0001 (Figure 1), and y = 0.0101x + 0.0002 for low and high concentrations, respectively.



(a)



(b)

Figure 1. Spectrophotometric linear calibration curve of TiO_2 for: (a) low concentrations and (b) high concentrations of TiO_2

Percent recovery

The percent mean recovery of TiO_2 from gelatin spiked samples was 95.74% ± 1.06% (Figure 2), which showed the applicability of the method to foodstuffs with high yield.

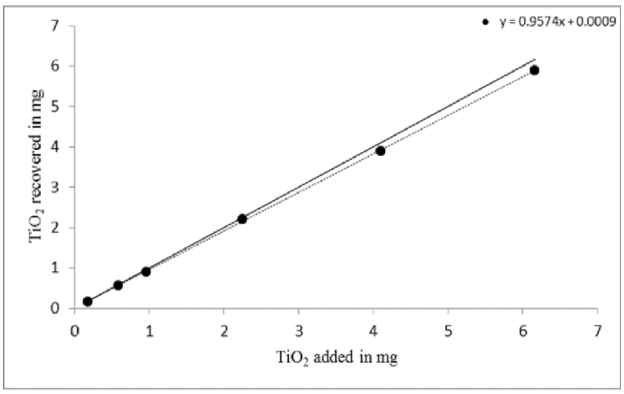


Figure 2. Recovery of TiO_2 in control samples suspended in gelatin. The % recovery = (the calibration slope ± standard deviation of slope) × 100%. The solid line presented by the equation (y = x) represents the 100% recovery

Standard addition

The standard addition calibration equation for the spiked 0.08 g Shampart juice portions was y = 0.0089x + 0.0603 with R^2 = 0.9999. The standard addition curve gave an intercept of 6.775 mg/L (Figure 3) which represents the TiO_2 concentration in the original sample as 8.469 mg/g or 0.847%. The external calibration curve result for the 0.15 g shampart juice showed 11.90 mg/L TiO_2 concentration corresponding to 0.1172 absorbance that represents the TiO_2 concentration in the original sample as 8.320 mg/g or 0.832 %. The percentage relative error was -1.77% (assuming the standard addition result as the true value) indicating the accuracy of the external calibration curves, and the negligible effect of matrix interferences.

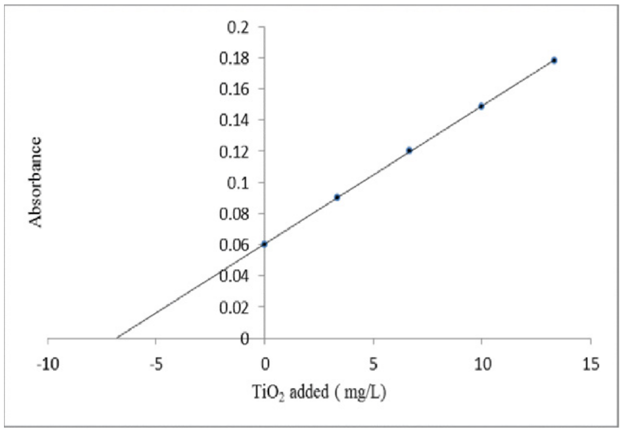


Figure 3.Standard addition curve of 0.08 g portions of Shampart juice powder

Titanium dioxide contents in foodstuffs

Results of TiO_2 content for all tested foodstuffs are summarized in Table 1. The results showed the absence of TiO_2 in Tahini, Halawa, canned humus, and liquid Jameed for all tested brands (absorbance’s values were zero); matching the manufacturer’s labels information, and complying with the Jordan Institution of Standards and Metrology. Regarding % TiO_2 content (± standard deviation) in different brands of gums and instant powdered drinks, it ranged from 0.019% to 0.91%, with deviations from 0.003 to 0.06 %; not exceeding the 1.0% limit by the FDA and compromising the presence of TiO_2 nanoparticles in these foodstuffs, making them safe to be consumed. The detection limit based on 3S/m (where S is the standard deviation (n = 7) for 1 ppm TiO_2, and m is the slope of the calibration curve for low concentrations) was 0.43mg/L TiO_2.

Titanium dioxide nanoparticles

Scanning Electron Microscope image (Figure 4) for the food grade E171 showed that most of the particles lie in ranges approximately 100 -150 nm,

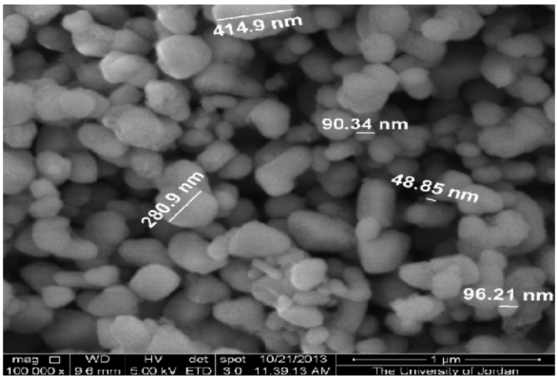


Figure 4. Scanning Electron Microscope image of E171 food grade. Most of the unlabeled particles are higher than 100 nm

and 150 - 300 nm or more, where a small fraction of the particles < 100 nm of approximately 15% was screened. Assuming that the TiO_2 nanoparticles is 36%, as mentioned before, and taking that the maximum TiO_2 content in our tested foodstuffs was 0.91% corresponding to 17.69 mg/1.94 g in mentos chewing gum, this will give us approximately 6.36 mg TiO_2 nanoparticles/ 1.94 g of piece of gum, and if we calculate per body weight of 60 Kg, it will be 0.055 mg/Kg body weight. The daily intake of TiO_2 nanoparticles of this gum based on four pieces per day will be 0.22 mg/Kg/day, other foodstuffs daily intake values are shown in Table 1. The calculated values are considered very small amounts to cause inflammation or cancer compared to the amounts used in animal studies to investigate TiO_2 nanoparticles health effects (Ramanakumar 2008 and Warheit 2007).

Conclusion

In this study, twenty five tested foodstuffs were analyzed for TiO_2 content. These stuffs were chosen because they are consumed in very large amounts in the Middle East on a daily basis. The optimized Myers' spectrophotometric procedure was used for evaluating the content of TiO_2 because of cost effectiveness, simplicity, and accuracy, with acceptable LOD results for TiO_2 opacity and whiteness in foodstuffs. The results showed that these foodstuffs complied with the Jordan Institution of Standards and Metrology for locally manufactured items, and FDA regulations for exported foodstuffs. The presence of TiO_2 nanoparticles was negligible in the tested foodstuffs that contained TiO_2, and below any limit that can cause cancer or mutation. Children should be offered natural gums or TiO_2 free gums.

Acknowledgments

We acknowledged the Chemistry department at the University of Jordan for providing research facilities, Prof. Manar Fayyad for her guidance, and Prof. Mario Monteiro for text editing.

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