Titanium dioxide content in foodstuffs from the Jordanian market: Spectrophotometric evaluation of TiO$_2$ nanoparticles

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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 different types of foodstuffs to ensure that limits did not exceed 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%.

Keywords

Foodstuffs, Titanium dioxide, TiO$_2$ nanoparticles, Myers’ procedure UV, Spectrophotometer, Wet digestion, Scanning electron microscope (SEM).

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 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.
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 TiO₂ 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 Schärchi), Copper sulfate CuSO₄ and Potassium sulfate K₂SO₄ (99%) were supplied by JHD fine chemicals, China. Titanium 1000 mg/L certified stock standard solution (NH₄)₂TiF₄ (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 H₂SO₄, 3.5 g K₂SO₄, and 0.40 g CuSO₄ at 310°C on a hot plate for one hour and a half, then adding 4.0 ml concentrated HNO₃, and 4.0 ml concentrated HCl gradually to the hot solution for another half an hour, where samples were covered by watch-glasses (Myers et al. used Kjeldahl digestion without HCl or HNO₃). 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% H₂O₂. 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 TiO₂ concentrations were calculated from the corresponding molecular weight ratio of TiO₂ 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 TiO₂ for low concentrations and 0, 2, 4, and 6 mg of TiO₂ for high concentrations, this was done to confirm reproducibility of the calibration curves using either solid TiO₂ 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 TiO²) 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. TiO₂ concentration results are compared with the same TiO₂ 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 TiO₂ 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 TiO₂ content according to the prescribed procedure above using the external calibration curves.

Scanning Electron Microscope
TiO₂ (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

TiO₂ calibration curves
Two linear TiO₂ 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. TiO₂ content in foodstuffs

<table>
<thead>
<tr>
<th>Food stuff item</th>
<th>Brand/Country</th>
<th>Portion size/g</th>
<th>TiO₂ mg/portion</th>
<th>% TiO₂/DI* (%)</th>
<th>DI: Daily intake in mg TiO₂ nanoparticles per body weight of 60 Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jamneed</td>
<td>Bent El. Bashar/ Jordan</td>
<td>A veggie serving for 1 persons/160 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Jamneed</td>
<td>Karak/ Jordan</td>
<td>A veggie serving for 1 persons/160 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Jamneed</td>
<td>Joodna/ Jordan</td>
<td>A veggie serving for 1 persons/160 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Jamned</td>
<td>Jameedha/ Zamar/ Jordan</td>
<td>A veggie serving for 1 persons/160 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Halawa with pistachios</td>
<td>Al-Nahlah/ Jordan</td>
<td>Average slice / 34 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Halawa with pistachios</td>
<td>Dura/ Jordan</td>
<td>Average slice / 34 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Halawa extra with pistachios</td>
<td>Asem/ Zamar/ Jordan</td>
<td>Average slice / 34 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
<tr>
<td>Halawa</td>
<td>Ayesh/ Jordan</td>
<td>Average slice / 34 g</td>
<td>&lt; LOD</td>
<td>E 171 is not included in the ingredients’ label</td>
<td></td>
</tr>
</tbody>
</table>

*LOD: Limit of detection.  
** DI: Daily intake in mg TiO₂ nanoparticles per body weight of 60 Kg

| Tahina                | Kasheh gold/ 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                | Selem/ Jordan               | Average tablespoon/5.00 g | < LOD           | E 171 is not included in the ingredients’ label |
| Canned hummus         | Beroni/ Jordan              | Average tablespoon/1.50 g | < LOD           | E 171 is not included in the ingredients’ label |

*LOD: Limit of detection.  
** DI: Daily intake in mg TiO₂ nanoparticles per body weight of 60 Kg

| Powdered juice Peach flavor | Shampar/Turkey | Pack/16 g | 183.29 mg | 0.83 | 0.05 | Ingredients label indicated the addition of TiO₂ (E 171) |
| Powdered juice Lemon        | Shampar/Turkey | Pack/16 g | 86.29 mg  | 0.50 | 0.05 | Ingredients label indicated the addition of TiO₂ (E 171) |
| Powdered juice Tang/ Egypt  | Pack/25 g      | 57.12 mg  | 0.25 | 0.01 | Ingredients label indicated the addition of TiO₂ (E 171) |
| Powdered juice Squez/ Syria | Pack/45 g      | 21.70 mg  | 0.48 | 0.003 | Ingredients label indicated the addition of TiO₂ (E 171) |

*LOD: Limit of detection.  
** DI: Daily intake in mg TiO₂ nanoparticles per body weight of 60 Kg

| Chewing-Gum Fused bubble/ Japan | Piece/4.2312 g | < LOD | Ingredients label indicated the addition of TiO₂ (E 171) |
| Chewing-Gum Sharaw/ Jordan      | Piece/ 1.5100 g | 0.24 mg | 0.017 | 0.003 | Ingredients label indicated the addition of TiO₂ (E 171) |
| Chewing-Gum Ticac/ Ireland      | Piece/ 0.5000 g | 0.44 mg | 0.09 | 0.005 | Ingredients label indicated the addition of TiO₂ (E 171) |

*LOD: Limit of detection.  
** DI: Daily intake in mg TiO₂ nanoparticles per body weight of 60 Kg
from TiO\textsubscript{2} or those with expected small amounts (low concentrations' calibration curve), while other products indicated the addition of considerable amounts of TiO\textsubscript{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.

**Percent recovery**

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

**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\textsubscript{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\textsubscript{2} concentration corresponding to 0.1172 absorbance that represents the TiO\textsubscript{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.

**Titanium dioxide contents in foodstuffs**

Results of TiO\textsubscript{2} content for all tested foodstuffs are summarized in Table 1. The results showed the absence of TiO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2}, and m is the slope of the calibration curve for low concentrations) was 0.43mg/L TiO\textsubscript{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,
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.

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### References


