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A Novel Approach for the Analysis of Titanium Dioxide Content by Simple Digestion System in Foodstuffs from the Egyptian Market Using ICP-OES and ICP-MS

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Abstract

Titanium dioxide (TiO_2) or E 171 is the naturally occurring oxide of titanium. It has been used as a colourant to improve and brighten the appearance of foods. Several toxicological studies have revealed that nanoparticles can cause inflammation, tissue necrosis, renal apoptosis, and immune response. Moreover, the International Organization for Cancer Research has listed TiO_2 as "possibly carcinogenic to humans''. Food labels do not provide quantitative data to the amount of TiO_2 added and even qualitative data is limited. In recent years, ICP-OES and ICP-MS have emerged as viable methods for the qualitative and quantitative determination of TiO_2**.** No ICP method had been reported for the determination of E171 colorant in Egyptian foodstuffs**.** In this study, rapid quantitative analysis was developed for determination of E171 in foodstuffs consumed widely by children. To efficiently recover E171, we combined concentrated sulfuric acid, nitric acid, and hydrogen peroxide with microwave digestion. The optimized digestion temperature was 200°C and digestion time was 15 min . ICP-OES was then used to analyse the digestate. ICP-MS was used as a complementary technique to ICP-OES to obtain assured results and achieve accuracy. A percentage recovery >95% for E171 was achieved. The method was successfully applicated on eleven foodstuffs found in the Egyptian market including chewing gums, hard candy, and juice powders.

Keywords: Titanium dioxide**;** ICP-OES**;** ICP-MS**;** Foodstuffs.

1. Introduction:

We are surrounded by titanium dioxide (TiO_2) containing materials which are used daily and may represent a risk on human health. Because of its high refractive index and extremely low solubility, TiO_2 accounts for nearly 70% of total global pigment production **(R.Baan, et al., 2006)** . Its use as a white pigment has steadily increased since 1916, and global production in 2018 exceeded 7 million metric tons. **(F.Loosli, , et al. 2019)** Due to these excellent physicochemical characteristics, It is widely used in a variety of applications to improve the quality and palatability of consumer products such as food, personal care products, cosmetics, paints, printing inks, paper, plastic goods, and coatings (**J.F.Jacobs, et al.,2010; K.Liu, et al. 2014; T.Rajh, et al. 2014; P.Łabuz, et al. 2013; Y.Yang, et al. 2014; F.Hong, et al. 2017; M.G.,Ammendolia, et al. 2017)**. Furthermore, TiO_2 has emerged as one of the top five nanoparticles used in consumer products over the last few decades **(R.K.Shukla,et al. 2011; J.Bai, et al. 2014).**

TiO_2 is a naturally occurring titanium oxide. It is known as titanium white, pigment white 6 (PW6), E171, or CI 77891 when used as a pigment. It is a white crystalline solid with superior chemical and physical properties to many other white pigments used in various fields of technology **(Dos Santos, et al. 2016).** It occurs as rutile, anatase, and brookite minerals. It has been used as a colorant in foods and personal care products such as candies, chewing gum, soft drinks, toothpaste, dietary supplements, and drug capsules to enhance and brighten the color **(K.Luo, et al. 2020).** Extending the use of E171 as a food additive in modern processed foods would significantly increase its oral intake regardless of consumer age or gender **(K.Luo, et al. 2020).**

Titanium dioxide is non-flammable and highly resistant to heat, light, pH, and oxygen, so it is unaffected by almost any food processing. It's also insoluble in water, most acids, and all organic solvents. However, it dissolves slowly in hot concentrated sulfuric acid (H_2SO_4) and hydrofluoric acid (HF) **(EC, Commission Directive 95/45. 1995).**

The combination of HF and HNO_3 was previously used in various studies for dissolving TiO_2 confined in samples in several experiments **(A.Weir, et al. 2012, R.J.Peters, et al. 2014).** Although HF has a superior ability to dissolve inorganic oxides, it is inconvenient for researchers' health because its chemical properties are highly toxic, corrosive, and difficult to handle. Furthermore, special equipment is required to analyze E171 when using HF as a digestion reagent, implying that HF is incompatible with general instruments equipped with glass components of ICP-OES and ICP-MS **(N.Kim, et al. 2018).**
Furthermore, many suggested digestion methods included the use of perchloric acid ($HClO_4$), which, due to its highly corrosive and oxidizing properties, is not available in most accredited industrial hygiene laboratories. Other commonly used alternative methods include the addition of nitric acid alone or in combination with sulfuric acid, which results in very low recoveries and incorrectly reported exposures.

In this recent study, we used a combination of concentrated sulfuric acid (95%), nitric acid (69%), and hydrogen peroxide (30%) with microwave digestion to recover E171 efficiently. Microwave acid digestion provides a safer platform for achieving higher local temperatures and pressures that can break down the lattice structure of TiO_2, which is required for accurate titanium quantification using ICP-OES or ICP-MS **(N.M.Hassan, et al. 1996).** As a result, it is clear that a convenient method for digestion assessment is required.

Microwave digestion was optimized for rapid quantitative analysis using ICP-OES and ICP-MS systems by modifying several conditions, including the acids used, digestion time, and temperature. As a result, the optimized digestion temperature of 200 ^{0}C with a mixture of concentrated H_2SO_4, HNO_3, and H_2O_2 resulted in a 95% recovery for E171.

We ran into two major issues while attempting to determine the dietary distribution of the food additive E171. First, food labels do not provide quantitative data and, even qualitative data, are of limited value, because food labelling regulations require additives to be identified at all times, unless they serve no additive function in the final product **(Ministry of Agriculture.2013)** Second, databases of food composition do not contain analytical data on food additives, but instead focus on nutrient data; additionally, manufacturers' data are constantly changing due to the use of different ingredients and recipes **(Ministry of Agriculture.2013)**.

A number of toxicological studies have revealed that E171 nanoparticles can cause inflammation, tissue necrosis, renal apoptosis, and immune response **(S.Gui, , et al. 2013; S.Bettini, et al. 2017; F.Grande and P. Tucci. 2016)** Moreover, the International Organization for Cancer Research has listed TiO_2 as "possibly carcinogenic to humans". In response to the growing health concern about the oral consumption of E171, France announced a ban on the use of E171-containing food products beginning in 2020 **(X.Audran and France Bans. 2019).**

Spectroscopic methods such as ICP-MS or ICP-OES belong to the most universal, sensitive, robust, and selective methods of detection and quantification of the total contents of metals. Several studies have reported E171 measurements in various matrices using a UV/VIS spectrometer **(W.Myers, et al. 2004)** , ICP-OES **(I.A.Mudunkotuwa, et al. 2016; M.C.Lomer, et al. 2000; W.van Bussel, et al. 2010; J.Boguhn, et al. 2009; Ş.Sunguret al. 2020)**, ICP-MS (**R.J.Peters, et al. 2014; F.Dutschke, et al. 2017; I-L.Hsiao, et al. 2016; .López-Heras, et al. 2014; Y.Picó, 2016; V. Golja, et al. 2017; P.Krystek, , et al. 2014; K.Khosravi, , et al. 2012)**, and single particle ICP-MS (**F.Laborda, et al. 2014; F.Laborda, , et al. 2019; J.Vidmar, et al. 2017)**.

In recent years, ICP-OES and ICP-MS have emerged as viable methods for the qualitative and quantitative determination of E171. The quality of ICP data for E171 analysis greatly depends on sample preparation, so sample digestion to a clear solution is critical for accurate food analysis using ICP-OES and ICP-MS systems.

A literature survey revealed that no ICP method had been reported for the determination of E171 colorant in Egyptian foodstuffs that children consume greatly. As a result, the goal of this research was to create a dependable, convenient, and efficient method based on a specific digestion system. The results obtained by this method demonstrates that a robust, simple, accurate, time-saving, and validated method for assessing E171 content in Egyptian foodstuffs, was achieved using microwave-assisted acid digestion. ICP-MS was used as a complementary technique to ICP-OES in order to obtain assured results and achieve accuracy.

2. Experimental

2.1. Materials & Reagents

We purchased Titanium (IV) oxide, anatase (99.8% purity), from the company Sigma-Aldrich (St. Louis, USA). All of the used chemicals were of analytical grade. We used the following Merck reagents (Darmstadt, Germany): sulfuric acid H_2SO_4 (95%), nitric acid HNO_3 (69%), and hydrogen peroxide H_2O_2 (30%). A Milli-Q system (Bedford, MA, USA) was used to deionize Ultra-Pure water (18 MΩ-cm).

Commercial products claiming to contain E171 colourant were purchased from various supermarkets.

A total of eleven foodstuffs including chewing gums, juice powders and hard candy were analyzed and reported in this study. A unique identification name was given to each product such as ***Q01- Q011***.

2.2. Sample preparation

To determine Titanium (IV) oxide concentration, food samples were first digested in an acid matrix by using a Topwave Analytik-Jena-UK microwave digester (maximum power: 1000 Watt). Approximately 0.5 gm. of each sample, 7 mL of HNO_3 (69%), 2 mL of H_2SO_4 (95%), and 1 mL of H_2O_2 (30%) were added into a digestion vessel. They were digested for 15 min at 200°C.

A standard solution of E171 was included in the digestion process. After microwave digestion, series of dilutions were performed to the working solution of E171 to attain the calibration range(0.01-10 µg mL^{-1}).

The vessel contents were placed in a 50-ml volumetric flask and makeup to the mark with ultra-pure water. All samples were then analyzed using an Agilent 5100 ICP-OES at 336.122 nm, followed by an 8800 ICP-MS-QQQ (Agilent Technologies). Calibration verification was prepared and analyzed along with the food samples. The molecular weight of TiO_2 was used to calculate a weight fraction of Ti, which was then used to calculate the percent (%) concentration in food samples. All ICP-OES and ICP-MS samples were analyzed in triplicate.

2.3. Microwave-assisted acid digestion system

Using a Topwave Analytik-Jena-UK microwave digester, food samples were first digested in an acid matrix. It has an excellent sample preparation capability for ICP analysis. Microwave sample heating results in rapid digestion and high throughput rates.

2.4. Inductively coupled plasma-optical emission spectrometry (ICP-OES)

All measurements were made on a synchronous Vertical Dual View (SVDV) Agilent 5100 ICP-OES Spectrometer with Dichroic spectral combiner technology (DSC), which allows ICP to detect a two-dimensional spectrum at the same time. It is supplied with a vertically orientated torch, which gives high matrix capability. Table 1 showed the operating conditions for the ICP-OES. A V-groove nebulizer and a reduced-volume Sturman Masters Type spray chamber comprise the sample introduction system.

Table 1: Operating conditions for the ICP-OES device

Parameter	Setting
• Viewing mode	Axial
• Radio frequency (RF) power	1.20 KW
• Torch	Vertically orientated
• Plasma flow rate	12 $Lmin^{-1}$
• Auxiliary flow rate	1 $Lmin^{-1}$
• Nebulizer flow rate	0.70 $Lmin^{-1}$
• Viewing height	8 mm
• Pump speed	12 rpm
• Replicates	3
• Replicate read time	5 sec.
• Instr. Stabilization delay	15 sec.
• Uptake delay	25 sec.
• Rinse time	0 sec.
• Spectral range	167-785 nm
• Analytical line	336.12 nm

When the solution passed through the orifice in the V-groove, it was nebulized. The spray chamber's inner surface is rough to ensure proper drainage. The instrumental software is responsible for determining a target integration time based on the intensity of the spectral line in order to achieve the optimum signal-to-background ratio (SBR).

2.5. Inductively coupled plasma-mass spectrometry (ICP-MS).

In order to obtain assured results and achieve accuracy, an 8800 ICP-QQQ with a PFA inert kit and nebulizer (Agilent Technologies, Santa Clara, CA) was used as a complementary technique to the ICP-OES. Table 2 depicted the operating conditions.

3. Results and Discussion

3.1. Digestion method optimization

Because TiO2 (E171) is one of the least soluble metal oxides, it must be completely dissolved in a solution before being quantified using ICP-OES and ICP-MS systems. This low solubility complicates E171 exposure assessments. Thus, To prepare samples for ICP-OES and ICP-MS analysis, powerful oxidising agents are required under extreme pH conditions.

To achieve complete recovery of results, microwave-assisted acid digestion was used in this current method. Methods like dry ashing and wet digestion were also performed, but those trials were not convenient for sample digestion and provided poor recovery. The factors affecting the microwave method of digestion were identified as acid matrix, digestion temperature and digestion time.

The digestion was carried out under various conditions, as shown in Table 3. The optimum conditions for E171complete dissolution were (acid matrix= 7 mL HNO3 (69%), 2 mL H2SO4 (95%), and 1mL H2O2 (30%); digestion time= 15 min; digestion temperature= 200 C). The digestate was then analyzed using ICP-OES and ICP-MS as mentioned previously.

3.2. ICP-OES analysis

3.2.1. Linearity and range

For E171, the ICP-OES instrument was calibrated using seven calibration solutions ranging from 0.01 to 10 µg mL^{-1}. The calibration yielded a correlation coefficient R^2, 0.9996 with good linearity, as shown in Table 4.

3.2.2. Limit of Detection (LOD) and limit of quantification (LOQ)

LOD was calculated as (3.3 × SD of the blank / calibration curve slope). LOD for E171standard solution was about 0.17 µg mL^{-1} while LOQ was calculated as (10 × SD of the blank / calibration curve slope). LOQ for E171 standard solution was about 0.52 µg mL^{-1}.

Table 2: Operating conditions for the ICP-MS device

Parameter	Setting
• RF-Power	1.50 KW
• Carrier gas flow	1.20 $Lmin^{-1}$
• Plasma gas flow	12 $Lmin^{-1}$
• Auxiliary flow	1 $Lmin^{-1}$
• Spray chamber temperature	2°C
• Spray chamber	Water cooled double pass
• Mass resolution	0.8
• Integration time points/MS	3
• Replicates	3

Table 3: Summary of digestion conditions included in the method optimization

Factor	Levels
• Acid matrix	▪ HNO_3 (100%), HNO_3: H_2SO_4 (1:1) ▪ HNO_3: H_2SO_4 (2:1), H_2SO_4 (100%) ▪ HNO_3:H_2SO_4:H_2O_2
• Digestion time (min)	40, 35, 20, 15
• Digestion set temperature(°C)	190, 250, 210, 200

Table 4: Characteristic parameters of the calibration equation for the determination of E171 using the ICP-OES method

Parameters	E171
Calibration range (µg mL^{-1})	0.01-10
LOD (µg mL^{-1})	0.17
LOQ (µg mL^{-1})	0.52
Regression Equation (Y)[a]: *Slope (b)	1.29×10^{5}
Standard deviation of the slope (S_b)	2.03×10^{3}
Relative standard deviation of the slope (%)	1.50
Confidence limit of the slope [b]	$1.16\times10^{5} – 1.41\times10^{5}$
Intercept (a)	6.43×10^{3}
Standard deviation of the intercept (S_a)	6.68×10^{3}
Confidence limit of the intercept [b]	$-7.95\times10^{3} – 2.08\times10^{4}$
Correlation coefficient (r)	0.9997

[a] Y= a+ bc, where c is the concentration of E171 in µg mL^{-1} and Y is the intensity

[b] 95% confidence limit

3.2.3. Precision and Accuracy

The accuracy of the method was assessed by analyzing E171 standards at two levels (0.05 and 0.5 µg mL^{-1}). Amounts of E171 found for the 0.05 and 0.5 µg mL^{-1} standards were 0.0503 and 0.511 µg mL^{-1}, while relative standard deviations (RSD %) were 1.5 and 0.84 %, respectively. RSD for food samples varied from 0.02 to 1.67 %.

3.3. Application to real food samples

After method validation, eleven Egyptian food products containing E171 were analyzed by ICP-OES, followed by ICP-MS. Then, the concentration of E171 (%) in each product was given in Table 5.

The range of E171 concentrations was between 0.001 % - 5.1 % (10 -51000 mg/kg).

The FDA established that the quantity of E171 should not exceed 1% by weight of the overall food. The concentrations of E171 in six food products were higher than the 1% legal limit, as shown in Table 5, whereas five products were within the legal limit.

Table 5: List of some selected Egyptian foodstuffs investigated in this study by ICP-OES and ICP-MS techniques

Sample ID	Category	Manufacturer	mg% TiO_2 concentration (Mean ± SD)		% RSD	
			ICP-OES	ICP-MS	ICP-OES	ICP-MS
Q01	Chewing gum	International Kallas company, Egypt	1.3±0.01	1.28±0.01	0.77	0.78
Q02	Chewy candy	PAMIR Gida San, Egypt	1.62±0.02	1.59±0.003	1.23	0.19
Q03	Juice powder	Mondelez Egypt Foods S.A.E.	1.67±0.003	1.64±0.01	0.18	0.61
Q04	Juice powder	Mondelez Egypt Foods S.A.E.	0.36±0.001	0.41±0.001	0.28	0.24
Q05	Chewing gum	Mondelez Egypt Foods S.A.E.	<0.001±0.000012	<0.001±0.000011	1.20	1.10
Q06	Chewing gum	Kallas international Co. for sweets, Ismailia, Egypt	2.75±0.03	2.68±0.03	1.09	1.12
Q07	Juice powder	Holw El-Sham Co., food indust. (A.S.E), Egypt	5.10±0.01	5.12±0.001	0.20	0.02
Q08	Juice powder	Holw El-Sham (A.S.E), October city , Egypt	2.00±0.001	2.01±0.003	0.05	0.15
Q09	Chewing gum	Baraka Co., food industries, Obour, Egypt	0.58±0.002	0.55±0.001	0.35	0.18
Q010	Hard candy	Advanced food industries, Gandour, Obour city, Egypt	0.06±0.001	0.06±0.001	1.67	1.67
Q011	Chocolate substitutes	Marbella, food industry S.A.E, October city, Egypt	0.81±0.01	0.84±0.005	1.24	0.59

4. Conclusion

Consumers, especially children, deserve to be safe without any risk factors in their feed. It is crucial; therefore, that the amounts of E171 in foodstuffs be closely monitored and maintained within stipulated limits. We have developed a convenient, simple, and efficient method for the determination of E171 in various Egyptian foodstuffs consumed widely by children. These products require a precise investigation to ensure safety. ICP-OES and ICP-MS techniques were applied to determine E171 concentration. Results were reliable and showed that not all commercial products were within the internationally stipulated limits. Therefore, some products need to be taken into consideration owing to their harmful effects on human health. To our knowledge, this study is the first work that reports the amounts of E171 in foodstuffs available in the Egyptian market.

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