Colorimetric Micro-determination of Titanium Dioxide in Foods

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A colorimetric procedure for the micro-determination of titanium dioxide (TiO_2) has been developed. The method incorporates wet-ashing and color development using diantipyrylmethane. The yellow-to-orange color is quite stable. Interference from Fe^{3+} ion could be removed in the presence of ascorbic acid. More than 90% of TiO_2 spiked at the 10 and 100 $\mu\text{g/g}$ levels were recovered from cheese and white chocolate. The proposed method is rapid, simple and accurate. The minimum level for determination of TiO_2 was 5 $\mu\text{g/g}$ when 2 g of sample was assayed.

Titanium dioxide (TiO_2) is frequently used as a whitener in cheese, chocolate and chewing gum. Current regulations of The Ministry of Health and Welfare of Japan have no limits for the use of TiO_2 in foods mentioned above. The use of the material and the absence of legal limits for its use initiated the work on a quantitative method to meet any regulatory demands.

A quantitative method was developed which incorporated ashing of the sample, dissolution of the ash in sulfuric acid to a known amount of volume, and development of yellow color with hydrogen peroxide¹⁾. The reaction is, however, of very low sensitivity, and is subject to interference from other ions²⁾. We therefore have sought for an alternative spectrophotometric method.

In the literature, titanium in minerals was determined spectrophotometrically by using organic reagents³⁾⁴⁾⁵⁾. However, these methods involve many steps for color development. Atomic absorption spectrometry also is an unfavorable method because of the difficulty in

atomization of titanium. Although a little less sensitive than other organic reagents³⁾⁴⁾⁵⁾, diantipyrylmethane is relatively specific for titanium and less subject to interference from other metallic ions⁶⁾. In the present work, we intended to make use of diantipyrylmethane for the quantitation of TiO_2 in foods.

Materials and Methods

Food samples

Processed cheese, chocolate and chewing gum were purchased from local market in Kobe City.

Reagents and apparatus

Reagent solution for color development reaction was prepared by dissolving 2.5 g of diantipyrylmethane (analytical grade, Wako Pure Chemicals) in 100 ml of 2.5 N HCl. Titanium dioxide (TiO_2) of high purity 3-N grade was obtained from Mitsuwa Pure Chemicals. All other reagents were of analytical grade.

Hitachi Model 200-10 spectrophotometer with 1-cm cells was used for measurements.

Preparation of standard solution

Accurately weigh 50 mg of TiO_2 and transfer to 500 ml tall beaker. Add 10 g of ammonium sulfate and 10 ml of concentrated H_2SO_4 . Cover with watch glass, and then heat the contents on hot plate to dissolve. Cool, and cautiously add 100 ml of distilled water with stirring. Cool, transfer the solution to 500 ml volumetric flask, and dilute to the mark with distilled water. The concentration of titanium of this solution is 0.1 mg/ml as TiO_2 . Dilute this solution with distilled water to make a series of working standard solutions (0.2 ~ 10 $\mu\text{g}/\text{ml}$ as TiO_2).

Preparation of sample digests

Take 0.5 ~ 2 g of food sample, depending on whether food is rich in sugar or not, into 300/ml Kjeldahl flask. Add 10 ml of water if sample is solid. Add 5 g of ammonium sulfate, 10 ml of concentrated H_2SO_4 and 5 ml of HClO_4 , then bring the contents to boil. If the digest fails to darken on prolonged boiling during destruction of organic matter, cool and add a few more drops of HClO_4 . Boil gently until evolution of dense white fumes of HClO_4 ceases. Cool the flask and contents, add 10 ml of distilled water, transfer the solution quantitatively to a 50 ml volumetric flask with an aid of distilled water, and finally dilute to the mark. The resulting 50 ml of digest solution, of which H_2SO_4 concentration did not exceed 7.2 N in maximum, should be colorless and free from suspended solids. Prepare a reagent blank solution at the same time from the same volumes of acid used in sample digestion.

Assay of titanium dioxide

Take 5 ml of sample digest solution containing not more than 50 μg of TiO_2 into a 25 ml test-tube. Add 1 ml of 5% (w/v) ascorbic acid solution and 4 ml of reagent solution in this order, and mix well. Allow the mixture to stand for 10 min at ambient temperature. Measure the absorbance of this solution at a wavelength of 390 nm against a blank solution in which 2.5 N HCl replaced the reagent solution. Calculate the TiO_2 in food sample from a calibration curve.

Results and Discussion

Effect of the amounts of reagent and reaction time

Titanium is well known to react with diantipyrylmethane to form yellow-to-orange color complex⁶. The spectra of complex show a well defined maximum at 390 nm. Hence, the measurements of absorbance were done at 390 nm in the subsequent experiments. No detailed information on the optimum amount of diantipyrylmethane is available. We therefore examined the effect of the amount of diantipyrylmethane on the reaction. If we assume that 3 μmol of diantipyrylmethane reacts with 1 μmol of titanium, then 146 μg of this reagent are sufficient for 10 μg of titanium (as TiO_2). However, the amount of diantipyrylmethane needed for optimum reaction under our analytical conditions was largely in excess of the theoretical value, as is obvious from Fig. 1. This is simply ascribed to the faster kinetics of the reaction with large amounts of reagent. Taking into account the cost of reagent and speed of analysis, 4 ml of diantipyrylmethane reagent (25 mg/ml) was adopted for routine analysis.

JAFFERY and GREGORY⁶) reported that more

Fig. 1 Effect of amount of diantipyrylmethane on the color development

Ten μg of titanium dioxide was reacted with varying amounts of diantipyrylmethane for 10 min.

than 2 h was required for maximum color development. However, under our analytical conditions 10 min was sufficient (see Fig. 2). This discrepancy can be explained by the fact that the amount of reagent used in this study was 4 times higher than that in the literature⁶⁾.

Effect of acidity

It is obvious from the literature⁶⁾ that concentration of HCl in the reaction mixture is a critical factor for the optimum reaction of titanium with diantipyrylmethane. Hence, the optimum concentration of HCl was examined. The maximum color development was obtained at HCl concentration of 0.7–1.3 N under our assay conditions. Accordingly, we made the reagent solution by dissolving diantipyrylmethane in 2.5 N HCl. The use of 4 ml of this reagent gave HCl concentration of 1 N in 10 ml of the reaction mixture. Concerning the effect of other mineral acids on the reaction, no detailed evaluation has been made. In this study, effect of H₂SO₄ concentration on the reaction was examined within 1–6 N. At these H₂SO₄ concentrations, the color intensity did not alter in response to change in acidity. This is beneficial to our assay system, in which the digest solution with high acidity was directly subjected to assay of titanium.

Fig. 2 Effect of reaction time on color development

Ten μ g of titanium dioxide was reacted with 100 mg of diantipyrylmethane for indicated time.

Digestion of food sample

Much has been written on the relative merits of dry and wet ashing for the determination of metal ions⁷⁾⁸⁾. We prefer wet ashing for the determination of TiO₂, because it normally requires less time and the risk of the occurrence of losses by volatilization is eliminated. When white chocolate spiked with 100 μ g/g of TiO₂ was digested using H₂SO₄ alone, the value obtained by the proposed method was only about 60% of the spiked level. In contrast, wet ashing with H₂SO₄ in the presence of ammonium sulfate (5 g) gave satisfactory results (99.5 μ g/g). From these observations, it is obvious that the presence of ammonium sulfate is essential for the determination of TiO₂. At present, we have no reasonable explanation about the effect of ammonium sulfate.

Perchloric acid was used as an oxidant rather than HNO₃ for two reasons. First, trace amounts of HClO₄, if retained in the digest solution, did not affect the reaction of titanium with diantipyrylmethane. Secondly, the complete destruction of organic matter required much less time when HClO₄ was used rather than HNO₃ was used in the presence of H₂SO₄.

Effect of diverse ions

Titanium (2 μ g/ml as TiO₂) was determined in the presence of a number of diverse ions (Table 1). Moderate amounts of various

Table 1 Effect of diverse ions on the determination of 2 μ g/ml of titanium dioxide

Diverse ion	Added as (μ g/ml)	Absorbance at 390 nm
None		0.42
Ca ²⁺	CaCl ₂ (200)	0.42
Mg ²⁺	MgCl ₂ (20)	0.42
Pb ²⁺	PbCl ₂ (20)	0.42
Fe ³⁺	FeCl ₃ (2)	0.49(0.42)*
Zn ²⁺	ZnSO ₄ (20)	0.42
Cu ²⁺	CuSO ₄ (20)	0.42
Al ³⁺	AlCl ₃ (20)	0.42
PO ₄ ³⁻	H ₃ PO ₄ (200)	0.42

* Ascorbic acid was added.

metal ions and anions commonly present in foods were tolerated. However, Fe³⁺ (2 µg/ml) interfered in the proposed method. Interference from Fe³⁺ was avoided by prior reduction to lower valency states with ascorbic acid.

Calibration curve

The calibration curve prepared according to the present method is linear. Beer's law is obeyed for titanium (as TiO₂) in the range 0.2 ~ 10 µg/g. The relation between absorbance (A) at 390 nm and the concentration (C) of TiO₂ was obtained by the least-squares method :

$$C = 4.77 A + 0.02$$

Therefore, the present method had a minimum quantitative level of about 5 µg/g when 2 g of sample was digested and diluted to 50 ml.

Recovery test

Processed cheese and chocolate, which are known and verified to be free of TiO₂, were selected for the preparation of sample to contain 10, and 100 µg TiO₂. Samples containing TiO₂ were prepared by making a slurry with hot water (60°C), adding known amounts of TiO₂, and blending in a Waring blender. The samples were then heated on a 60°C water bath and periodically mixed by stirring to release the added water and to return the samples to their approximate original weight and consistency. The samples were then analyzed according to the method described in Materials and Methods. Recovery of TiO₂ from 2 kinds of foods were satisfactory, results being 92.1 ~ 94.9% for spiking at 10 µg/g and 97.4 ~ 99.8% for spiking at 100 µg/g (see Table 2).

Application to commercially available foods

Table 3 shows a comparison of results obtained for the determination of TiO₂ in several commercially available foods using the proposed method and hydrogen peroxide method¹⁾. The latter method gave somewhat lower values compared to those obtained by the proposed method, especially for food sample low in TiO₂ contents. This may be attributed to low sensitivity and/or complex procedure for sample treatment in the hydrogen peroxide

Table 2 Results of recovery study of titanium dioxide in cheese and chocolate

Food	Amount added (µg/g)	Recovery* (%)
Processed cheese A	10	92.1±2.3
	100	97.4±2.4
Processed cheese B	10	94.9±3.1
	100	99.3±2.6
White chocolate A	10	93.8±1.8
	100	99.5±2.8
White chocolate B	10	93.7±2.6
	100	99.8±1.9

* Results represent mean value and standard deviation of triplicate determinations.

Table 3 Comparison of methods on the determination of titanium dioxide in several kinds of foods

Food	Titanium dioxide content(µg/g)	
	Proposed method*	Hydrogen peroxide method
Processed cheese A	N.D.**	N.D.**
Processed cheese B	N.D.**	N.D.**
White chocolate	3 200±85	2 950
Marble chocolate (brown)	15±0.4	N.D.**
Marble chocolate (yellow)	50±2.1	35
Chewing gum	N.D.**	N.D.**

* Results represent mean value and standard deviation of triplicate determinations.

** N.D.: Not detected.

method.

In summary, the proposed method is rapid and reliable. Further, the sensitivity of this method is more than 20 times higher than that of hydrogen peroxide method. Ten to 20 samples can be assayed routinely for TiO₂ in 5 ~ 6 h as opposed to 1 or 2 days when hydrogen peroxide method is used.

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食品中の二酸化チタンの微量比色定量法

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食品中の二酸化チタンの微量定量法について検討した。試料 (0.5~2 g) を硫酸アンモニウムの存在下で濃硫酸と過塩素酸で湿式分解し、冷後蒸留水で 50 ml に定容した。これの 5 ml を比色管にとり、1 ml の 10% アスコルビン酸と 4 ml のジアンチピリメタン溶液 (25 mg/ml 2.5 N 塩酸) を加え、30 分室温で放置後 390 nm で吸光度を測定する。二酸化チタンの標準溶液を試料と同様に操作して得られた検量線より試料中の二酸化チタン量を求めた。チーズおよびホワイトチョコレートに添加した二酸化チタンの回収率は、10 µg/g で 92.1-94.9%, 10 µg/g で 97.4-99.8% であった。

なお、本法の定量限界は、試料 2 g を用いた場合 5 µg/g であった。

新 刊 紹 介

卵の調理と健康の科学

佐藤 泰・田名部尚子・中村 良・渡辺乾二共著

本書は、食形態と健康に重点をおいて解説した鶏卵の参考書であり、その内容は、① 卵の成分と物性、② 殻付卵の品質と貯蔵法、③ 卵の調理と機能、④ 卵料理の活用、⑤ 卵製品の活用、⑥ 卵の栄養と健康とで構成されている。家政学、畜産学、農芸化学における指導書としてだけでなく、食品工業に携わる技術者、研究者にとって有益な書である。

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