WebLog Journal of Analytical and Pharmaceutical Chemistry
Research Article
Published: 20 Oct, 2025

Iodometry and UV Spectrophotometry Determination of Iodine in Some Selected Salt Samples and Freshly Harvested Fish Samples

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Abstract

Iodine is a chemical element with the symbol I and atomic number 53. It is a micro nutrient required for proper functioning of the vertebrate endocrine system and plays similar roles in numerous other organisms. The recommended daily intake by WHO-UNICEF is between 90 mg/kg to 250 mg/kg in adults. The aim of the work is to determine the Iodine concentration in selected freshly harvested fish species namely Sole fish (*Solea Solea*), White Catfish (*Ameiurus Catus*), Croaker fish (*Scianidae*) and Red Snapper fish (L*utjanus Campechanus*), and six commercial edible salts in Nigeria using two techniques which are UV/ Vis spectrophotometry and Iodometry. The spectroscopic method is based on the reaction of iodate (in acidic medium) and iodide to form iodine and an aqueous matrix was used. For the salt analysis, the analytical signal was measured at 588 nm using the UV-Visible Spectrophotometer. The result of the Visible spectrophotometric for Iodine concentration gave a result in the range of (38.16 to 1189.69) mg/kg while the Iodometric titration gave a result in the range (5.36 - 29.17) mg/kg. The analytical curve was linear in the range of 10-60 mg/L (10-60 mg/kg) with a correlation coefficient of 0.9912 which shows that the data fits into the model. Comparing this technique with the titration reference method in the six commercial table salts, a significant difference was observed applying paired t-test at 95% confidence interval. The proposed method is a simple, economic and a reliable alternative for iodine species determination of salt. For the fish analysis, alkaline based ashing was used for the sample preparation. The result obtained from the UV/VIS spectrophotometry shows the iodine concentration to be in the range of 200.7 mg/kg to 517 mg/kg while the Iodometry result shows the iodine concentration to be in the range of 85.90 mg/kg to 427.59 mg/kg. a paired t-test was used to evaluate the significant difference between the two methods and no significant difference was observed at 95% confidence level. It therefore follows that the two methods can be used for analyzing iodine content in fish samples. Furthermore, the result obtained shows that the fish species are good sources of iodine for man.

Keywords: Iodine; Iodometry; Ultra-Violet Spectrometry (UV Spec); Fish; Salt

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Received Date: *10 Oct 2025*
Accepted Date: *17 Oct 2025*
Published Date: *20 Oct 2025*

Citation:
Fatunsin O. T, Dosunmu O. M, Fasanya F. O. Iodometry and UV Spectrophotometry Determination of Iodine in Some Selected Salt Samples and Freshly Harvested Fish Samples. WebLog J Anal Pharm Chem. wjapc.2025.j2003.

Introduction

Iodine is an essential trace element in biological systems. It has the distinction of being the heaviest element commonly needed by living organisms as well as the second-heaviest known to be used by any form of life. It is a component of biochemical pathways in organisms from all biological kingdoms, suggesting its fundamental significance throughout the evolutionary history of life [12].

The human body only needs 150 micrograms (or 20,000th of a teaspoon) of iodine to meet up with the standard set for daily requirements and not more than a teaspoon full in the whole lifetime (Delange *et al*., 2001). Major target organs are the developing brain, muscle, heart, pituitary gland, and kidney (Ahmed *et al*., 2008). Iodine is also a major element that determines the health of three connective tissues in fetuses (Johnson *et. al*., 2013). Parkinson's disease, multiple sclerosis and Alzheimer's disease have also been linked with iodine deficiency (International Medical Veritas Association, 2011). Consumption of minerals such as magnesium, manganese, calcium, and fluoride can restrict the absorption of iodine uptake by the thyroid gland. Excess intake of iodine prevents proper production of thyroid hormones leading to iodine induced hyperthyroidism with similar manifestation to IDD (Vania *et al*., 2001). Iodine deficiency in pregnant women can lead to neurocognitive deficiency in the children. Stunted growth, emotional emptiness, impaired speech and hearing problems may also develop as a result of this deficiency (Zimmermann *et al*., 2013).

Diets deficient in iodine increase risk of retarded brain development in children (cretinism), goitre (Erica *et al*, 2009) mental retardation (Erica *et al*, 2009; Hetzel *et al*., 2000), high cholesterol (Stephen *et al*., 2006), fatigue and depression (Ebert *et al*., 2008; Stephen *et al*., 2006), lethargy (Hetzel *et al*., 2000), and weight gain.

Oceans are worldwide repository of iodine (Patrick *et al*., 2008), very little of earth iodine can actually be formed in the soil. Iodine has uneven distribution in the earth. In water, it is found in organic and inorganic forms (seawater/ocean) and also in soil. Iodine is formed from iodide ions in sea water which are oxidized, volatilized and evaporated into atmosphere and returned into soil by rain (Zimmmerman *et al.,* 2008). Iodine content in the soil is affected by planting, fertilizer and irrigation. Marine foods have higher concentration of iodine than other foods since marine animals concentrate iodine from seawater (Zimmmerman *et al*., 2008). Iodine is rich in some food groups: fish, marine and shellfish, dairy products (yoghurt and quark milk and cheese), vegetable and fruits (apple, banana, avocado, tomato, egg plants and cashew). Cereal (white and brown bread, cornbread, biscuit and cake) also contain iodine (Haldimann *et al*., 2005).

Iodine in Food

Iodide, iodate and elemental iodine can undergo oxidation and reduction cycles in a food system. For example, iodine reactions within the food system may affect the bioavailability of iodine as a fortificant from the time of iodization to consumption. However, there is very little published information detailing possible iodine reactions and their impact on food systems. Historically, iodine has been added as a fortificant to salt and most literature focuses on the use of iodized salt as a replacement for non-iodized salt in processed foods. The possibility of fortifying foods directly with iodide or iodate justifies a wider examination of the chemistry and reactivity of iodine and its salts in foods.

Iodine Chemistry

Iodine is the heaviest, naturally occurring member of the halogens. Elemental iodine (I_2) does not occur in a stable form in nature and the two most common naturally occurring forms are iodide (I^-) and iodate (IO^{3-}). Iodine does not occur in a stable form in nature, it is an important intermediate in iodine reaction chemistry.

The standard half-reactions for iodate and iodine are:

$2IO_3^- + 12H^+ + 10e^- \rightarrow I_2 + 6H_20$ $E^o = -1.194V$

$I_2 + 2e^- \rightarrow 2I^-$ $E^o = 0.536V$

These indicate that both iodate and elemental iodine are oxidizing agents and iodide is a reducing agent. These two equations can be combined to give:

$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_20$

Iodide can be oxidized to elemental iodine by oxygen or other oxidizing agents, especially in the presence of catalysts, such as metal ions and moisture. The reaction between iodine and oxygen is very slow in neutral pH, but is rapid in acidic solution. The reaction is accelerated by sunlight and catalysts such as nitrite and cupric ion (Cu^{2+}). In a humid, acid environment, and in the presence of oxidizing agents, iodide is readily oxidized to iodine, which is volatile (Louis *et al*., 2007).

Elemental iodine readily sublimes and is rapidly lost to the atmosphere through evaporation and diffusion Iodate can be reduced to elemental iodine by a variety of reducing agents. However, the most common outcome of oxidation reactions with iodate is the formation of iodide. These reactions are dependent upon pH and ionic strength. In summary, elemental iodine does not occur naturally but it is a key intermediate in iodine reaction chemistry. Potassium and sodium iodates are strong oxidizing agents, while the iodide salts are reducing agents. As such, they are potentially important reactants in food systems. The chemistry of the reversible inter-conversion of iodate to iodide involves elemental iodine and thus a study of iodine and its salts in food systems must take into account all three forms.

Methods of Analysis

Chemistry of Iodometric Method of Analysis: Most of the methods for determining the iodine in iodized salt involve oxidation of the iodide to iodate, acidifying, addition of potassium iodide and titration of the liberated iodine with Standardized Thiosulphate.

$5I^- + IO^{3-} + 6H^+ \rightarrow 3I_2 + 3H_2O$ … Equation (1)

In the presence of a strong acid, iodide reacts with iodate to generate iodine, as shown in the equation Eq (1).

$I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^- + 3H_20$….. Equation 2

Liberated iodine can be titrated with thiosulphate. According to the equation Equation (2), the consumed amount of thiosulphate is proportional to the amount of free iodine liberated from the salt.

Spectrophotometric Method of Analysis: Spectrophotometry is a method used to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. It can also be used to measure the amount of a known chemical substance. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

Experimentation

Samples Collection: Various brands of commercially available salts were purchased, specifically: Mr Chef, Dangote, Bajara, Uncle Pam, and Finest. Also measured salt usually displayed in buckets and measured was obtained. The fish samples are selected freshly harvested fish samples which were purchased from the popular Oyingbo Market in Lagos State, Nigeria. The fishes are Sole fish (*Solea Solea*), White Catfish (*Ameiurus Catus*), Croaker fish (*Scianidae*) and Red Snapper fish (*Lutjanus Campechanus*).

Reagents and Instruments: Analytical grade reagents were used in the analysis. They are: Potassium Iodide, Potassium Iodate, Sulphuric Acid, Starch, Potassium Dichromate. Sodium Hydroxide, Sodium Thiosulphate, Sodium Trioxocarbonate (IV), Methanol

Instruments:

Instruments used in the study are:

- UV/Visible Spectrophotometer
- Desiccator
- Analytical Balance
- Oven
- Furnace

Preparation of Stock Solutions and Reagents

Stock Solutions were prepared for the project and the working solutions were prepared by serial Dilution.

- Stock Solution of Potassium Iodate 1000ppm: Potassium Iodate salt of 1.22 g of was dissolved in 800 mL Standard Flask and made up to 1L. Other working Solutions were prepared from the Stock Solution by serial dilution.
- Stock solution of Potassium Iodide 1000ppm: Potassium Iodide salt of 1.046 g was dissolved in 800 mL Standard Flask and made up to1L. Other working Solutions were prepared from the Stock Solution by serial dilution.
- Preparation of 10% KI: Potassium Iodide salt of 10 g was dissolved in 80 mL of water and made up to 100 mL in the standard Flask.
- Preparation of 10% KIO_3: Potassium Iodate salt of 10 g was dissolved in 80 mL of water and made up to 100 mL in the standard Flask.
- Preparation of 1% Starch Solution: Starch powder of 1g of starch was weighed and added to 100 mL of water and allowed to boil.
- Preparation of 0.005 M Sodium Thiosuphate: Sodium Thiosulphate salt of 0.079 g was weighed and added to 80 mL of water and then made up to 100 mL
- Preparation of 0.001 M Potassium Dichromate: Potassium Dichromate salt of 0.024 g, after oven dried at 180°C, was weighed into 80 mL of water then made up to 100mL in the Standard Flask.
- 1M Sulphuric Acid: Concentrated Sulphuric acid of 5.4 mL was measured and added to 80 mL of water and then made up to 100 mL.

Preparation of Standards for Calibration Curve: Standard Solutions of Potassium Iodate and Potassium Iodide were prepared from freshly prepared Stock Solutions and absorbance taken and used for external Calibration.

Salt Analysis

Procedures for the Quantification:

Quantitative determination of Iodine species in Iodized Table Salts: The presence of iodide and iodate species in iodized salts was determined according to the procedure below as described by Kabani below (Kabani *et al*., 2002.).

Spectrophotometry determination of Iodate (IO^{-3}): Firstly, 2 g of salt was dissolved in 10 ml of distilled water in a test tube. Then 1 ml of 10 % potassium iodide (KI) solution and 2 ml of 1 M sulfuric acid were added. Then few drops of 1 % starch solution was added and checked for the blue coloration. Then Centrifuged to clear solution before quantitative determination of Iodate at 588 nm using UV/Visible Spectrophotometer.

Spectrophotometry Determination of Iodide (I^-): In the case of iodide (I^-), first 2 g of salt was dissolved in 10 ml of distilled water in a test tube. Then 1 ml of 10 % potassium iodate (KIO_3) solution and 2 ml of 1 M sulfuric acid were added. Then few drops of 1 % starch solution was added and checked for the blue coloration analyzed at 651nm.

Procedure for determination of moisture content of the salts: The moisture content of each salt sample was determined using the oven drying technique. Approximately about 10 g of salt sample dried at 105 ^{0}C for 2 hours and the moisture content calculated using the difference of initial and final weights of the sample.

$$\% \text{ Moisture content } \frac{weight\ of\ wet\ salt - weight\ of\ dry\ salt \times 100}{weight\ of\ wet\ salt}$$

Moisture Content = Initial Weights – final Weight

For calculating the iodine content on dry basis, the moisture content subtracted from the weight of salt taken for the iodine Estimation. Using the equations Eq (3) and Eq (4), the iodine content of salt samples calculated on dry basis as I.

1 mL of 0.005 M $Na_2S_2O_3 \equiv 0.1058$ mg of iodine …… (3)

Iodine content of Salt as I, on dry basis (in mg/kg)

$$= \frac{\text{Consumed volume of Na2S2O3 in mL} \times 0.1058\ \text{mg/mL} \times 100\ \text{g/kg} \times 250}{\text{Dry weight of the salt sample in g} \times 50\ \text{mL}} \ldots (4)$$

Iodometric Titration

Procedure for the Standardization of Sodium Thiosulphate: Sodium Thiosulphate Solution was standardized with Standard Potassium Dichromate solution to determine its exact Concentration. 0.005 M Sodium thiosulphate was titrated with 25 mL 0.001M Potaasium Dichromate. 2 mL of I M Sulphuric acid and 5 mL of 10% KI was added to the titrand and kept in the dark for 5 minutes and then titrated with the titrant till the solution becomes pale yellow in colour then 1mL 1% starch solution was added. The solution turned dark blue and titrant was added until the solution turned colorless.

Procedure for quantitative determination of Iodine by Iodometric Titration: Accurately about 50 g of salt sample weighed and transferred into a 250 mL volumetric flask using deionized water. The sample dissolved well and made up to the mark, and 50.00 mL aliquot of the solution pipetted out into a stoppered flask. To this solution, 2 mL of 2 N sulfuric acid and 15 mL of 10% potassium iodide solution added. The flask stoppered immediately, shaken well and kept in dark for 10 minutes. The liberated iodine was titrated with 0.005 M sodium thiosulphate solution, with freshly prepared starch as an external indicator. The test duplicated for each sample. A blank test carried out using 50 g of AR grade sodium chloride (K abani *et al*., 2002).

Iodine content of salt as I, on dry basis (mg/kg) was calculated using the formula

$$I = \frac{consumed\ vol\ of\ Na2So3\ in\ ml \times 0.1058 mg/ml \times 100 g/g \times 250}{dry\ weight\ of\ the\ salt\ in\ g \times 50 ml}$$

Fish Analysis

Sample Preparation: Four fish samples of Sole fish (*Solea Solea*), White Catfish (*Ameius Gatus*), Croaker fish (*Scianidae*) and Red Snapper fish (L*utjanus Campechanusru*) were prepared as described by Pilar (Pilar *et al*., 200l). About 6 g of each fish was placed in three different big crucibles and 30 g of Na_2CO_3, 10 mL of a 6-M NaOH and 75 mL methanol were added. The crucibles were allowed to dry slowly to avoid analyte lose in an oven at 105 ^{0}C for 2 hours. After that, the crucibles were placed in a muffle furnace whose temperature was slowly increased to 450 ^{0}C to prevent analyte lose for approximately 3 hours for ashing.

Test for Iodine Presence: About 0.5 g of prepared sample was dissolved in a 2.5 mL of distilled water in a test tube. Then 025 mL of 10% KIO_3 solution and 0.5 mL of 1 M sulfuric acid were added. Then, two drops of 1% starch solution was added and checked for blue colouration. (ECSA-HC, 2007)

$IO_3^- + 5I + H_3O^+ \rightarrow 3I_2 + 9H_2O$

$I_2 + I^- \rightarrow I_3^- + Starch \rightarrow Blue\ Complex$

Test for Iodate Presence: About 0.5 g of prepared sample was dissolved in a 2.5 mL of distilled water in a test tube. Then 0.25 mL of 10% KI solution and 0.5 mL of 1 M sulfuric acid were added. Then 2 drops of 1% starch solution was added and checked for blue colouration. (ECSA-HC, 2007)

$5I^- + IO_3^- + H_3O^+ \rightarrow 3I_2 + 9H_2O$

$I_2 + I^- \rightarrow I_3^- + Starch \rightarrow Blue\ Complex$

Iodometry Determination of Iodine: About 10 g of food sample was dissolved in 50 mL distilled water. Then 2 mL of 2 N sulfuric acid and 5 mL of 10% potassium iodide were added to it. On shaking, the solution turned to a yellow colour. The flask was closed with stopper and kept in the dark for about 10 minutes. The samples were removed from the dark and titrated against the sodium thiosulphate solution until it turned into a very light yellow colour (pale yellow). Subsequently, a few drops (1-5 mL) of 1 % starch solution were added. The solution turned into a deep purple colour. Thiosulphate was added drop by drop from the burette until the solution became colourless and the final reading was taken. (De-Maeyer *et al,* 1979). The equation for the titration reaction is

$S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + I^-$

$I_2 + I \rightarrow I_3^-$

$I_3 + 2e^- \rightarrow I_3^-$

$2S_2O_3\ 2- + I_3^- \rightarrow S_4O_6^{2-} + 3I^-$

The iodine content concentration of the sample liberated is calculated out using the formula:-

$$\frac{C_1V_1}{C_2V_2} = \frac{n_1}{n_2}$$

Where C_1 = Concentration of the iodine to be determined

V_1 = volume of the sample

C_2 = Concentration of the thiosulphate used

V_2 = Volume of the thiosulphate used

n_1 = combining ratio of the iodine

n_2 = combining ratio of the thiosulphate

The Iodine concentration in the Iodide added before the titration was calculated and subtracted from the Iodine content obtained in the result to get the actual concentration of iodine in the sample.

Standardization of $Na_2S_2O_3$: The $Na_2S_2O_3$ solution is to be standardized with standard KlO_3 solution to determine its exact normality. 0.005 N $Na_2S_2O_3$ was put in a burette and pipette out 2.5 mL of 0.005 N standard H_2SO_4 and 5ml of 10% KI Solution. The solution was titrated against $Na_2S_2O_3$ till the solution became pale yellow in colour. To this, 10ml of starch was added. The solution turned deep purple. Thiosulphate solution was added drop by drop from the burette till the purple colour completely disappeared. (Josef, 2014)

Volume of KlO_3 (V_1) x normality of KlO_3 (N_1) = Volume of $Na_2S_2O_3$ (V_2) x normality of $Na_2S_2O_3$

Therefore, Normality of $Na_2S_2O_3 = \frac{V \times N_1}{V_2}$

UV Analysis of Iodine

Iodide Determination: About 1 g of each fish sample was dissolved in 5 mL of distilled water in a test tube. Thereafter, 1mL of 10% potassium iodate solution and 2 mL of 1 M sulphuric acid were added. Then few drops of 1% starch were added for colour formation and absorbance was taken on the UV Spectrophotometer.

The result obtained is in mg/kg. This is multiplied by 5 (which is the volume (mL) of the distilled water in which the sample was dissolved in) and the result divided by 1 (which is the gram (g) of sample dissolved). This is necessary in order to get the actual concentration of the Iodide in the amount of grams being analyzed.

Iodate Determination: About 1g of each fish sample was dissolved in 5 mL of distilled water in a test tube. 1 mL of 10% potassium iodide solution and 2 mL of 1 M sulphuric acid were added. Then few drops of 1% starch were added for colour formation and absorbance was taken on the UV Spectrophotometer.

The result obtained is in mg/kg. This is multiplied by 5 (which is the volume (mL) of the distilled water in which the sample was dissolved in) and the result divided by 1 (which is the gram (g) of sample dissolved). This is necessary in order to get the actual concentration of the Iodate in the amount of grams being analyzed.

The result obtained gives the concentration of Iodate and Iodide in the fish samples. The concentration of Iodine in the fish samples is calculated out from the Iodate and Iodide concentration obtained from the UV analysis using the formular:

$$\frac{M_1}{M_2} \times mg/kg$$

Where

M_1 = Molar mass of Iodine

M_2 = Molar mass of Iodate or Iodide

mg/kg = Concentration of Iodate or Iodide obtained from UV analysis.

Results and Discussion

Salt Analysis

Moisture Content of the Salt: Table 3.0 shows the moisture content of the salt samples. Finest Salt has the highest moisture content followed by Mr Chef salt and Uncle Pam salt has the least moisture content (Table 1).

Standardization of 0.005 M Sodium Thiosulphate: Standardized Sodium thiosulphate of 0.005 M was titrated against Potassium Dichromate.The Actual concentration of Sodium Thiosulphate is 0.004M.

Result of Titrimetry Determination of Iodine in Salt Samples: In table 1, the result from the salt analyses showed a sharp decrease

Table 1: Result of the Moisture Contents of the Salt Samples.

Table Salt Sample	Moisture Content
Mr Chef	0.24
Dangote	0.22
Bajara	0.11
Finest / Cassava	0.69
Uncle Pam	0.09
Measured/Unpackaged	0.28

Table 2: Result of the iodometric titration of the salt samples.

	Mr Chef	Dangote	Bajara	Finest	Uncle Pam	Measured Salt
Average Titre	34.30	17.40	8.40	7.30	9.50	6.30
Iodine content in mg/kg	29.17	14.79	7.12	6.26	8.05	5.36

Table 3: RDI, UIL, RDI.

Individual	Recommended daily intake (RDI) (mg)	Upper intake level (UIL) (mg)	Recommended daily intake mg/100g (RDI)
Infants ∠12 months	110	130	11.00-13.00
Little children (8-13 years)	90	-	9.00
Adults above 18 years	150	1,110	15.00-110
Pregnant women	220	-	22.00
Lactating mother	290	-	29.00

in Iodine concentration compared with 50ppm Iodate fortification recommended by SON and other regulatory bodies for the manufacturing Industry. This sharp decrease is due to storage and transportation of the salts. The results were lower than 150 mg daily recommended intake by (Lyday *et al*., 2000). The lower values obtained is also due to poor sensitization of the health benefit of iodine, volatile nature of iodine, improper handling of salt by the household. The values are also lower than the 100 mg dietary trace minerals according to (Berbel *et al*., 2007). The human body needs 150 mg of Iodine to meet up with the standard set for daily requirement (Delange *et al*., 2001). There is a need to take sea foods rich in iodine to avoid hypothyroidism (Table 2).

According to lyday *et al*. (2000), none of the salt samples analyzed with iodometric titration meet with these recommendations. Hence, there is possibility of hypothyroidism if the individual depends on the table salts as the only source of iodine in the diets to meet up with daily intake recommendation. There is a need to support with other iodine rich foods in order to meet up with these recommendations (Table 3).

Determination of Iodine By UV / Visible Spectrophotometric Method

Result of the calibration curve: Six Calibration Standards were prepared; they are 10, 20, 30, 40, 50, 60ppm. The calibration curve gave a slope of 0.0032, intercept of 0.0118 and a good correlation value of 0.9912 which shows that the regression model fit into the obtained values as shown in figure 1.

UV/Visible Spectrophotometric Results: The Analysis was carried out using GENESYS 180 UV /Visible Spectrophotometer at 588nm. Table 4 shows iodate concentration of the salt samples, actual concentration of the iodate calculated using the mass to volume ratio, then Iodine concentration of the salt samples were also calculated. Uncle Pam, Mr Chef and Dangote salts contained higher values greater the 50 ppm as recommended by the SON and other regulatory bodies as well as the iodine values (unicef *et al*., 2008) except Bajara and Finest salts. This may be due to the strategy employed by the manufacturers in order to meet up with the standard set by the regulatory bodies to add higher than recommended value because of the volatile nature of iodine. The iodine concentration of finest salt is lower than the recommended value for the manufacturer (unicef *et al*., 2008).

Higher Iodine values could also be due to random or systematic

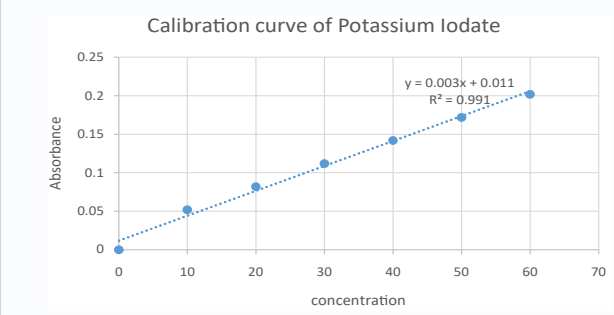

Figure 1: Calibration Curve of Iodate Standards.

Table 4: Result of UV/Visible Analysis of Iodate salt samples.

Salt sample	Absorbance	Iodate Concentration form calibration curve (mg/L)	Iodate Concentration from calibration curve including mass and volume used for analyses (mg/Kg)	Iodine concentration (mg/kg)
Chef	0.129	29.29	146.45	86.91
Dangote	0.048	17.83	89.15	52.90
Measured	0.159	40.00	200.00	118.69
Uncle Pam	0.134	31.07	155.35	92.19
Finest	0.083	12.86	64.30	38.16
Bajara	ND	ND	ND	ND

errors. Errors could cause the measured values to be too high or too low. It could be a systematic error that is, the instrumental errors.

Higher values of iodine concentration per day greater than the recommended value can lead to toxicity of iodine which also depends on the amount of food taken (Anette *et al*., 2010). The daily intake for a normal adult is 100-150 microgram per day and in the presence of goitrogens, the intake should be increased to the range of 200-300 microgram per day (Lisbeth *et al*., 2004).

Iodine should be taken in moderate recommended amount in order to avoid hyperthyroidism or hypo-thyroidism as the case may be (Table 4).

Iodide Determination of The Salt Samples: Iodide determination in table salt gave a negative result because Salt is now fortified with a stable potassium iodate instead of unstable potassium Iodide (Unicef *et al*., 2008). There was no visible colour observed.

Application to Salt Samples

In order to evaluate the applicability of Visible Spectrometric method for Iodine Determination, six commercial table salts were analyzed with visible method and with the conventional Iodometric Titration. The table 5 shows the results obtained for the Iodine Content from the two procedures, expressed as mg/kg of salt are shown below. The application of Paired t-test to the obtained data with the two methods showed that there was significant difference at 95% confidence level ($t_{experimental} = 3.07$, $t_{critical} = 2.44$) but at 99.9% confidence interval $t_{experiment}$ is lower than t_{Tab}, there is no significant difference (Table 5).

Fish Analysis

Result and Discussion: Calibration curve for four different standards for the Iodate and iodide was obtained using the UV

Table 5: Comparison of the two methodologies.

Sample	Iodine concentration in Iodometric Titration Method (mg/kg)	Iodine concentration (mg/kg) in Visible spectrophotometric Method
Mr Chef	29.17	86.91
Dangote	14.79	52.90
Measured	5.36	118.69
Uncle Pam	8.05	92.19
Finest	6.26	38.16
Bajara	7.12	ND

Table 6: Absorbance of Standard Obtained from the UV Instrument for Iodate and Iodine Standards.

Iodate Standard	Absorbance	Iodide Standard	Absorbance
25 ppm	0.1990	25 ppm	0.005
50 ppm	0.0720	50 ppm	0.041
75 ppm	0.0930	75 ppm	0.096
100 ppm	0.0142	150 ppm	0.130

Thermo Scientific brand, BIOMATE 3 Model, made in the United States of America (USA). The absorbance for the concentration at each standard of the Iodate and Iodide as obtained from the instrument for is shown in table 6.

The graphs of the calibration curve for the Iodate and Iodide standards are shown in figure 2 and figure 3.

The equation for the calibration curve obtained for the Iodate was $y=0.0001x - 0.016$ ($R^2 = 0.976$) while the equation obtained for the Iodide was $y=0.0001x - 0.004$ ($R^2=0.880$). The concentration of the Iodate and Iodide calculated in the grams (g) of samples analyzed and expressed in µg/g (section 3.7.1 and 3.7.2) after it was obtained from the calibration curve graph. This is shown in table 7.

The Iodate concentration in the fish samples, with the exception of the Croaker fish (*Scianidae*), is observed to be lower than the Iodide concentration sequentially. The iodide is greater than the Iodate in the respective fish samples.

From the Iodate and Iodide concentration obtained, the Iodine content in each fish sample was calculated by using the formular explained in earlier section shows the Iodine in Iodate and Iodide of each fish sample as well as the total iodine present in each fish.

Iodine content in the fish samples had a range of 200.7 µg/g to 517.0 µg/g. This is obtained from the addition of iodine calculated from Iodate and Iodide. The contribution of Iodine from the Iodide is greater than that from the Iodate. This underscores the research works

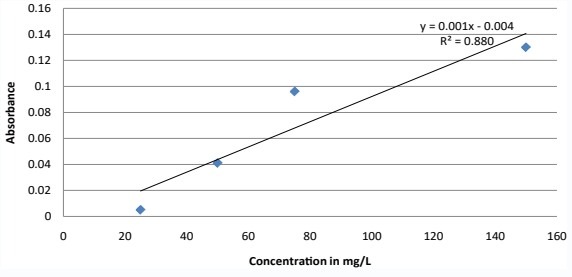

Figure 2: Absorbance of Standard obtained from the UV Instrument for Iodide.

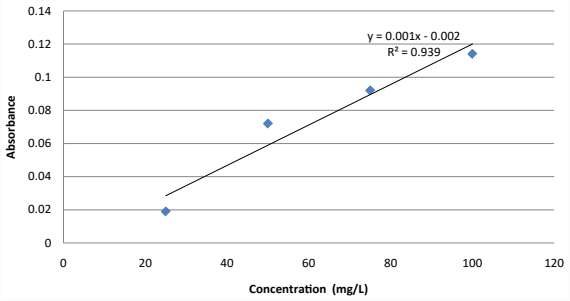

Figure 3: Absorbance of Standard obtained from the UV Instrument for Iodate.

done by scientists that human Iodine bioavailability from Iodide is higher than from Iodate (Li *et al.*, 2012). Furthermore, according Milczarek, Iodide, and not Iodate is the potential protective agent against oxidative damage to membrane lipids in porcine thyroid (Milczarek *et al.,* 2013). This literally explains the reason why there is higher concentration of Iodide than Iodate by nature in the fish samples. Consequently, there is more contribution of Iodine from the Iodide than the Iodate (Table 8).

Result of Iodometry Analysis of Iodine in Fish Samples: The concentration of Iodine in the four fish samples as obtained from Iodometry analysis is shown in table 9.

The concentration of Iodine in the fish samples obtained from the Iodometry Analysis are slightly lower than the result obtained from UV analysis in the respective fish samples. This can be attributed to certain limitations inherent in the titrimetry method which include errors due to parallax, possibility of not having a complete reaction between the thiosulphhate and the analyte (Samples), amongst other human errors prone to tritimetry (Table 9).

Comparing of Results Of UV Analysis and Iodometry

The Iodine concentration in the fish samples obtained by UV Analysis and Iodometry Analysis are compared and shown in the table 10.

A paired t-test was used to evaluate the significant difference on the obtained as follows:

See Table 11.

$$\bar{d} = 138.27$$

$$sd = \sqrt{\frac{45888.78}{3}} = 123.67$$

$$\therefore \text{t-test} = \frac{\bar{d}}{sd}\sqrt{4}$$

$$= \frac{138.27}{123.67}\sqrt{4}$$

$$= 2.22$$

From the paired t-test calculated, it was observed that at 95% confidence level, ^{t}calculated < ^{t}tabulated. Therefore, there was no significant difference between the two methods and so both methods can be used for the analysis of iodine in fish samples. However, the UV method, being an experimental method, is preferable to the Titrimetry method which is a classical method because the UV method helps to overcome the limitations of the titrimetry method which include time saving, accuracy of result, use of less reagents and samples.

Table 7: Concentration of Iodate and Iodide of fish samples obtained by UV analysis.

Fish Samples	Iodate Absorbance	Iodate Concentration (Mg/L) From Calibration Curve	Iodate Concentration (Mg/Kg)	Iodide Absorbance	Iodide Concentration (Mg/L) From Calibration Curve	Iodide Concentration (mg/kg)
Sole fish (*Solea Solea)*	0.019	21	105	0.11	114	570
White Catfish (*Ameiurus Catus*)	0.059	61	305	0.085	89	445
Red Snapper fish (*Lutjanus Campechanus*)	0.028	30	150	0.026	30	150
Croaker fish (*Scianidae*)	0.068	70	350	0.034	38	190

Table 8: Iodine content of each fish sample.

Fish Samples	Iodine in Iodate (mg/kg)	Iodine in Iodide (mg/kg)	Total Iodine (mg/kg)
Sole fish (*Solea Solea)*	61.7	341.5	403.2
White Catfish (*Ameiurus Catus*)	179.3	337.7	517.0
Red Snapper fish (*Lutjanus Campechanus*)	89.2	112.5	200.7
Croaker fish (*Scianidae*)	205.8	144.2	350.0

Table 9: Iodine Concentration of Fish Samples Obtained by Iodometry.

Fish Sample	Iodine Concentration (mg/kg)
Sole fish (*Solea Solea)*	427.59
White Catfish (*Ameiurus Catus*)	256.4
Red Snapper fish (*Lutjanus Campechanus*)	85.09
Croaker fish (*Scianidae*)	148.59

Table 10: Concentration of Iodine in Fish samples obtained by UV Analysis and Iodometry.

Sample	UV Analysis (mg/kg)	Iodometry (mg/kg)
Sole fish (*Solea Solea)*	403.2	427.59
White Catfish (*Ameiurus Catus*)	517.0	256.54
Red Snapper fish (*Lutjanus Campechanus*)	200.7	85.09
Croaker fish (*Scianidae*)	350.0	148.59

Table 11:

S/No	UV Method	Iodometry	d	$(d-\bar{d})$	$(d-\bar{d})^2$
1	403.2	427.59	-24.39	162.66	26458.27
2	517.0	256.54	260.46	122.19	14930.39
3	200.7	85.09	115.51	-22.66	513.47
4	350.0	148.59	201.41	66.14	3986.65

Conclusion and Recommendation

A reliable, low cost and simple method by uv/visible spectrophotometric determination of iodate in salt was used for this research work. The procedure showed good accuracy and precision when compared to the other method. The requirement of sample size and reagent volume are small. Another remarkable characteristics is the simple UV-Visible equipment can be also considered as portable instrumentation and allows the acquisition by laboratories with reduced financial support.

The Iodine levels of these salts are higher than the 50 ppm as against the standards set for the manufacturer except for the finest salts. This is a strategy to make up for the volatility of Iodine species as 20% iodine is lost during production and 50% iodine is lost during packaging, storage and heat (Louis *et al*., 2007). This strategy used by the manufacturer can lead to hyperthyroidism if strict measures are not put in place. Iodine deficiency leads to hypothyroidism (Koumourou *et al*., 2002), the risk of hypothyroidism increases with age and more common in women than men.

The recommended WHO-UNICEF-ICDO daily intake of iodine is 90 mg/kg for preschoolers, 120 mg/kg for school children, 150 mg/kg for adolescent and 250mg/kg for pregnant and lactating women (Garcia Arrona *et al*., 2017). This therefore follows that of the four fish samples, the best source of iodine for a full grown adult is the Sole fish (*Solea Solea*), while the Red Snapper fish (*Lutjanus Campechanus*), does not give as much as the other fish samples. However, the four fish species are good sources of iodine for the body.

As a result of these factors that affect iodine retention, it is therefore recommended that there is a need to include sea foods in the diets because they have higher iodine concentration since marine animals concentrate iodine from sea water (Zimmermann *et al*., 2008) to meet with the 150 mg of iodine required to meet up the standard set for daily requirement.

The paired T-test showed that there is no significant difference between the two methods used for the analysis of iodine in the fish samples. Therefore, the two methods are reliable methods for the determination of Iodine in fish samples. However, titrimetry is the most frequently used method to determine quantities of Iodine (especially in salt samples) because of its accuracy, relatively easy to use and incurs low cost (Khazan *et al*., 2013).

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