



East, Central and Southern Africa Health Community
Fostering Regional Cooperation for Better Health

Laboratory methods for fortified foods
Part I

Determination of Iodine in salt by Iodometric Titrimetric Method

Reference Method

Second Edition 2017

Foreword

ECSA-HC has been working with partners in direct response to resolutions of the Conference of Health Ministers to scale up Food Fortification initiatives as a critical strategy in managing micronutrient malnutrition among populations of the member states.

Part of the outcome of the intensified collaborative initiatives, was publication of three parts of the first edition ECSA-HC manuals on laboratory methods of fortified foods namely part 1 on determination of iodine in salt, part II on determination of vitamin A in sugar and oils and Part III on determination of iron, vitamin A and riboflavin in fortified flours. The manuals have been implemented in the last 10 years.

During the food fortification workshop held in Arusha-Tanzania in September 2015, three working groups through which the ECSA-HC capacity building Initiative co-implemented by ECSA-HC and GAIN and supported by USAID were formed:

- i) Production, Food Safety and Quality Assurance/Quality Control.
- ii) Inspection and Enforcement; and
- iii) Consumption Monitoring and Program Impact. The groups were tasked with identifying capacity and resource gaps and propose ways of filling these gaps in each of the technical areas. Subsequently, they

identified priority activities, targets, and developed road maps on how the activities would be implemented to achieve the set targets. Target 2 of the Inspection and Enforcement Working Group was to review the Regulatory Monitoring Frameworks used by countries in the Region. To inform this review, a workshop was organized for this group at the Imperial Resort Beach Hotel in Entebbe, Uganda from the 7th to 10th November, 2016. Its aim was to review the existing guidelines (that countries are using) for gaps and weaknesses, and use recommendations from this review to develop harmonized and practical guidelines that all countries can adopt and apply in inspection of fortified foods and specialized nutritional products. A key recommendation of the Entebbe workshop was that the inspection manuals be merged and be developed into two guidelines namely internal and external monitoring of fortified foods and that of commercial and points of entry inspection guidelines. The same meeting recommended that the test methods be reviewed by the laboratory working group which was previously a sub-working group of the inspection and enforcement working group and had become the fourth working group.

ECSCA-HC with technical support from GAIN and financial support of USAID hosted a regional food fortification workshop for laboratory analysts between 13 – 16th December 2016 in Nairobi, Kenya that recommended review of the manuals to update of any new updates in the reference methods, format them as

guided by ISO/IEC directive 2 and presenting them as a simple test method format for use by the laboratories.

The first editions of ECSA manuals of laboratory methods are recognized as primary reference materials and have guided the development of this edition. In addition, reference has also been made to the latest editions of ISO, AOAC and Codex standards and duly recognized under the bibliography.

This part of revised test method is meant to directly contribute to the overall effort to strengthen food fortification in the region.

It is our hope that the use of this test method will help strengthen food control activities in our countries in order to deliver safe and quality fortified foods to the ECSA-HC population.

DIRECTOR GENERAL.

ECSA-HC

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Disclaimer

The content of these guidelines can be adapted to suit country specific contexts. In such a case, the content of the resulting document will be the sole responsibility of the organization adapting the guideline and will not represent the views of ECSA-HC. The Use of the content of these guidelines should be duly acknowledged.

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Acronyms and Abbreviation

AOAC	Association of Official Analytical Chemists
ECSA – HC	East, Central and Southern Africa – Health Community
GAIN	Global Alliance for Improved Nutrition
ISO	International Organization for Standardization
ISO/IEC	International Organization for Standardization/International Electrotechnical Commission
SWOT	Strength, Weakness, Opportunities and Threats
USAID	United States Aid for International Development

Introduction

Analytical methods of fortified food products are critical components of successful implementation of set standards and/or regulations by both the industry as well as the food control regulatory authorities.

The success of internal, external, commercial and points of entry monitoring, inspection and audit heavily relies on the accuracy, reproducibility, sensitivity and complexity of the methods to release reliable results of collected samples for action.

It is recommended that both industry and regulatory agencies use similar method in determining compliance to avoid possible disputes of the results. However, these methods are reference and therefore other validated methods as recommended in the annex may be used. It is also important to note that for routine internal monitoring industry may choose to use either qualitative or semi quantitative methods in monitoring but they should perform quantitative analysis at determined intervals. This part of test method therefore has provided reference method for determination of iodine in salt fortified with potassium iodate which is the recommended compound due to its stability. The test method has also provided; as an informative annex an alternative method which can test iodine presence from both the iodide and iodate fortified salts. It is recommended that analysts should determine the appropriate method to apply based on the national regulations on the form of iodine compound to be used.

DETERMINATION OF IODINE IN SALT

1.0 Scope of the method

This ECSA-HC method of determination of iodine in salt provide a reference method for the determination of iodine in which potassium iodate has been used to fortify salt by titration method.

The method does not apply where other forms of iodine compounds have been used in fortification such as potassium iodide. However, annexed to this method as **annex A** is the Codex recommended method for general testing of iodine in either iodate or iodide form. Other methods which may be used are listed in **annex B** of this test method.

2.0 Normative references

ECSA – HC (2007) guidelines on food fortification, 1st Edition.

ECSA – HC (2007) Manuals on internal and external monitoring of fortified foods 1st Edition.

ECSA – HC (2007) Food Control Manuals for commercial monitoring and points of entry inspection 1st Edition.

3.0 Terms and Definitions

3.1 Accuracy

Is the capacity of the analytical method to determine the amount of the analyte as close as possible to the true value.

3.2 Laboratory sample

Is a sample as prepared (from the lot) for sending to the laboratory and intended for inspection or testing.

3.3 Lot

Is the quantity of product that is assumed to be of the same production process and represented by specified sampling rules.

3.4 Precision

Is a general term for the variability among repeated tests under specified conditions.

3.5 Sensitivity

Is defined for the purpose of this manual as the degree of certainty that an analytical method can differentiate between two very similar amounts of the analyte or the smallest amount of substances in a sample that can accurately be measured by a method

3.6 Qualitative analysis

Is method used to determine the presence or absence of a nutrient and is ideal for screening samples to determine if the samples are fortified with targeted micronutrient.

3.7 Semi-qualitative analysis

Is method mainly used to monitor the micronutrient levels in the finished product during the fortification process at the factory. These methods are based on their respective qualitative methods, but are adapted to introduce comparative assessment based on intensity of color development or spot density.

3.8 Quantitative analysis

Is method which accurately determines the concentration (amount) of micronutrients in the food.

3.9 Test sample

Subsample or sample prepared from the laboratory sample and from which test portions will be taken

3.10 Test portion

Quantity of material drawn from the test sample (or from the laboratory sample if both are the same

4.0 Principle

This iodometric titrimetric method is used to determine the quantifiable amount of iodine in salt fortified with the potassium iodate.

Acid is added into a salt solution to make it slightly acidic followed by addition of excess KI. The iodate in the salt reacts with iodide (I^-) to form iodine (I_2) and triiodide (I_3^-), which dissolves in water to form a yellowish solution. When a starch solution is added, a blue colored complex between triiodide and starch is formed. The amount of iodine in solution is determined by a titration with a standard thiosulfate solution, which removes the iodine and as result the blue color disappears. The end point is visually determined by the disappearance of the blue color from solution when no more iodine is present.

5.0 Reagents

WARNING — Analysts should take into account the relevant national laws/ regulations on handling hazardous substances as appropriate as well as ensuring that technical, organizational and personal safety measures are adhered to.

Unless otherwise specified, use only reagents of recognized analytical grade.

5.1 Sodium thiosulfate, Prepare a 0.005 N by dissolving 1.24 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (FW= 248) in one liter of distilled water and store in a cool dry place this solution is stable for one month

Note: The thiosulfate solution shall be standardized by titrating with a standard 0.005N potassium iodate solution. This standard solution is prepared from a 0.5 N solution(s) made by dissolving 4.4585 g analytical grade KIO_3 in water and making up to 250mL. The 0.5 N solution(s) is diluted 100-fold by taking 2.5 mL and diluting to 250 mL with distilled water. Normality of the thiosulfate solution = [volume KIO_3 (sol)/ volume thiosulfate (sol)]/ x m Normality of iodate solution (0.005 N)]

5.2 Starch solution, prepared to 1 % solution

Note 1: Weigh 1 g of soluble starch and mix with enough cold water to make a thin paste, top up with to 100 mL with boiling water and boil while stirring to dissolve. The starch solution should be prepared daily.

Note 2: The weight could be reduced to less than 1 g, while maintaining 1 % solution, depending on the number of samples to be analyzed.

Note 3: If using a vitex starch note 1 does not apply but a pinch is added to the titration solution to prepare the solution

5.3 Potassium iodide solution, prepare a 10 % solution by dissolving 100 g of potassium iodide in water and make up to one liter. Store in a cool dark place. It should be prepared on daily basis

5.4 Sulphuric acid solution, Prepare a 2 N acid solution by slowly adding 60 mL of concentrated sulfuric acid to 900 mL of distilled water. The solution is cooled down and made up to one liter.

6.0 Apparatus

6.1 Beakers, use the beakers of the volume between 250 mL to 500 mL

6.2 Volumetric flask, 100 mL, 250 mL, 1 L

6.3 Erlenmeyer flask, 200 mL

6.4 Graduated cylinder, 50 mL

6.5 Pipettes, 1 mL, 5mL and 50 mL

6.6 Burettes, able to measure 10 mL to 50 mL

6.7 Weighing scale, calibrated to weigh approximately 50 g.

Note: Containers, calibrated by volume to weigh approximately 50 g of salt may be used instead.

6.8 Glass rods

6.9 Spatulas

6.10 Pipette fillers

6.11 Stop watch

7.0 Sampling

A representative sample whose integrity has been maintained during transportation and storage should be sent to the laboratory.

Sampling is not part of the method specified in this ECSCA – HC method. A recommended sampling method is given in the ECSCA – HC guidelines for internal and external monitoring of fortified foods, 2nd edition.

8.0 Preparation of the test portion

A test portion should be a representative sample of the laboratory sample well composited to ensure that a good homogeneity. It is from this composited test sample where test portion shall be drawn.

9.0 Procedure

9.1 General

Store the sodium thiosulfate standard solutions in amber bottles.

9.2 Preparation of test solution

9.2.1 Weigh accurately 50 g of salt in a beaker and dissolve it with distilled water by stirring with glass rod and transfer it to 250 mL volumetric flask and top up with distilled water.

9.2.2 Transfer 50 mL of the salt solution using a 50 mL pipette to a 200 - 250 mL Erlenmeyer flask.

9.2.3 using a graduated pipette add 1 mL of the 2N sulfuric acid to the salt solution and mix thoroughly.

9.2.4 Add 5 mL of the 10% potassium iodide solution using a measuring cylinder or a pipette. If iodine is present a yellowish solution is formed. Cover the flask and put it in the dark or cupboard for 10 minutes.

9.3 Titration of the prepared sample

9.3.1 Fill the 50 mL burette with the sodium thiosulfate solution.

9.3.2 Titrate the prepared salt solution in the Erlenmeyer's flask with 0.005N sodium thiosulfate while agitating continuously and stop the titration when the deep yellow colour of the solution turns to pale yellow.

9.3.3 Add 2 mL of the starch solution and mix thoroughly. The solution should turn blue/black.

9.3.4 Resume titration with thiosulfate while agitating continuously and gently until the blue/black color disappears.

9.3.5 Record the used volume as accurately as possible to the nearest 0.1 mL

10.0 Calculations

Iodine in mg/Kg shall be calculated as shown in the equation below:

$$\frac{0.005 N Na_2S_2O_3 \times Vol Na_2S_2O_3 (mL) \times 21.222 (g, eq I) \times 250 (mL)}{0.05 kg \times 50 (mL)}$$

This equation can be simplified to:

$$10.61 \times \text{volume of titer of } Na_2S_2O_3$$

Annex A: (Informative) – Codex Method for Iodine

The Codex Committee on Method of analysis (CCMAS) through Codex Stan 234 on method of analysis recommends EuSalt/A002 – 2005 method as a type II method for testing Iodine. The method is adopted here for ease of reference.

Reference of adoption: EuSalt/AS 002 – 2005

1. Scope and Field of application

The present EuSalt Analytical Standard describes a titrimetric method for the determination of total iodine (iodides and iodates) in sodium chloride. The method is applicable to products of iodine content (expressed as I) equal to or greater than 3.5 mg per kilogram of salt.

2. References

H. Furrer, M. Staub, Mitteilungen aus dem Gebiet der Lebensmitteluntersuchungen und Hygiene (1953), 44, 252

Gemeinschaftarbeit, Mitteilungen aus dem Gebiet der Lebensmitteluntersuchungen und Hygiene (1964), 55, 43

3. Principle

Dissolution of the sample in water

Oxidation of iodide to iodate with bromine water and elimination of the excess bromine with formic acid

Addition of phosphoric acid and potassium iodide with formation of free iodine equivalent to the amount of iodate present

Titration of free iodine with sodium thiosulphate using starch as indicator

4. Reagents

Unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

4.1. Phosphoric acid, $\rho \approx 1.7 \text{ g/ml}$, 85 % (m/m)

4.2. Formic acid, $\rho \approx 1.2 \text{ g/ml}$, 90 % (m/m)

4.3. Potassium iodide solution, $\beta_{\text{(KI)}} \approx 100 \text{ g/l}$

Prepare this solution on the day of use and store it in a dark bottle.

4.4. Bromine water, saturated at ambient temperature

4.5. Hydrochloric acid, $c_{\text{(HCl)}} = 0.1 \text{ mol/l}$

4.6. Sodium thiosulphate, $c_{\text{(Na}_2\text{S}_2\text{O}_3)} = 0.1 \text{ mol/l}$, standard volumetric solution

4.7. Sodium thiosulphate, $c_{\text{(Na}_2\text{S}_2\text{O}_3)} = 0.01 \text{ mol/l}$, standard volumetric solution

Prepare this solution by dilution of the standard volumetric solution (4.6.).

Standardize with a potassium iodate solution, $c_{\text{(1/6 KIO}_3)} = 0.01 \text{ mol/l}$.

4.8. Methyl red, 0.5 g/l solution in 95 % (v/v) ethanol

4.9. Starch solution, 2 g/l

Prepare this solution at the time of use from soluble starch.

5. Apparatus

Usual laboratory equipment and:

5.1. Burette allowing the distribution and measurement of 0.01 ml

5.2. 500 ml conical flask with ground stopper

6. Sampling and Samples

A test sample of 500 g should be taken for analysis, ensuring it is representative of the whole batch.

7. Procedure

7.1. Test portion

Weigh, to the nearest 0.1 g, about 50 g of the test sample.

7.2. Test solution

Transfer the test portion (7.1.) and 175 ml of water into a 500 ml conical flask (5.2.). Stir to dissolve.

7.3. Blank solution

Transfer 175 ml of water into a 500 ml conical flask (5.2.)

7.4. Determination

Proceed with the conical flasks prepared in (7.2.) and (7.3.) in the following way:

- Add 4 drops of methyl red (4.8.) and hydrochloric acid
- 0.1 mol/l (4.5) to the first colour change from yellow to orange and then add immediately 1.5 ml of bromine water (4.4). Allow to stand for 3 minutes
- Add some glass beads, heat and keep boiling for 5 minutes, with swirling and avoiding crystallization of sodium chloride.
- Allow to stand for 1 minute, add 1.0 ml of formic acid (4.2.) in such a way that the whole of the inside surface of the conical flask is wetted and swirl
- After 1 minute, cool to about 20 °C, add 1.0 ml of phosphoric acid (4.1) and 1.0 ml of potassium iodide solution (4.3). Swirl, cork the conical flask and allow to stand in the dark for exactly 5 minutes
- Titrate with the sodium thiosulphate standard volumetric solution 0.01 mol/l (4.7) using a burette (5.1). When the solution is nearly discolored, add 1 ml of starch solution (4.9) and continue the titration until the blue colour disappears for at least 3 seconds

8. Expression of results

8.1. Evaluation

The iodine content of the sample, $\omega_{(I)}$, is given by the formula:

$$\omega_{(I)} = 21.15 \times C(\text{Na}_2\text{S}_2\text{O}_3) \times \frac{1000}{m} \times (V_1 - V_0)$$

Where,

$\omega_{(I)}$ is the total iodine content, expressed as milligrams of iodine per kilogram of salt,

m is the mass, in grams, of the test portion (7.1),

V_1 is the volume, in milliliters, of sodium thiosulphate (4.7) used for the titration of the test solution (7.2),

V_0 is the volume, in milliliters, of sodium thiosulphate (4.7) used for the titration of the blank solution (7.3),

$c(\text{Na}_2\text{S}_2\text{O}_3)$ is the molar concentration of the sodium thiosulfate standard volumetric solution (4.7)

The result is expressed in a single decimal place

8.2. Repeatability and reproducibility

Analyses, carried out on six samples by 14 laboratories, have given the following statistical results, each laboratory having furnished results obtained by the same operator performing three analyses per sample:

Product	$\omega_{(I)}$	k	P	N	s_r	S_R
Rock Salt	24.4	13	13	3	0.77	1.55
Sea Salt	11.6	13	13	3	0.65	1.05
	63.2	12	12	3	1.19	3.08
Vacuum Salt	4.6	13	13	3	0.34	0.50
	15.2	13	13	3	0.49	0.70
	45.1	12	12	3	1.11	1.41

Where:

- $\omega_{(I)}$ is the total iodine content, in mg l/kg,

- k is the number of analysts,

- p is the number of laboratories retained after eliminating outliers,
- n is the number of results per series,
- s_r is the repeatability standard deviation, in mg of I/kg,
- s_R is the reproducibility standard deviation, in mg of I/kg.

Reference: European Committee for the Study of Salt, ECSS/ CN 172-1978, Statistical evaluation of the Inter-laboratory Study of Br, I, K, Ca, Mg, F.

8.3. Limit of quantitation

The limit of quantitation (LOQ = 3.5 mg I/kg) is based on the formula:

$$\text{LOQ} = 10 \cdot s_r$$

Where s_r is the repeatability standard deviation of a representative test sample having an iodine concentration near the expected LOQ (see Vacuum salt 4.6 mg I/kg).

This calculated value corresponds with a volume of titrant

$$[c_{(\text{Na}_2\text{S}_2\text{O}_3)} = 0.01 \text{ mol/l}] \text{ of } 0.8 \text{ ml.}$$

9. Remarks

9.1. For iodine content greater than 20 mg per kilogram of salt, reduce the test portion (7.1.) accordingly.

9.2. The presence of oxidizing agents may lead to inaccurate results. The Fe^{3+} interference can be avoided by complexation with EDTA.

9.3. An automatic titrator provided with a platinum electrode and an Ag/AgCl reference electrode may be used. In this case, do not add starch solution (4.9) during the determination (7.4).

Annex B: (Informative) List of other validated test methods

AOAC Official Method 925.56, Iodine in Iodized salt, Titrimetric Method, Vol 1:2016.

Bibliography

AOAC Official Method 925.56, *Iodine in Iodized salt, Titrimetric Method*, Vol 1:2016.

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