

Stability of iodine in salt fortified with iodine and iron

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Abstract

Background. Determining the stability of iodine in fortified salt can be difficult under certain conditions. Current methods are sometimes unreliable in the presence of iron.

Objective. To test the new method to more accurately estimate iodine content in double-fortified salt (DFS) fortified with iodine and iron by using orthophosphoric acid instead of sulfuric acid in the titration procedure.

Methods. A double-blind, placebo-controlled study was carried out on DFS and iodized salt produced by the dry-mixing method. DFS and iodized salt were packed and sealed in color-coded, 0.5-kg, low-density polyethylene pouches, and 25 of these pouches were further packed and sealed in color-coded, double-lined, high-density polyethylene bags and transported by road in closed, light-protected containers to the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), Delhi; the National Institute of Nutrition (NIN), Hyderabad; and the Orissa Unit of the National Nutrition Monitoring Bureau (NNMB), Bhubaneswar. The iodine content of DFS and iodized salt stored under normal room conditions in these places was measured by the modified method every month on the same prescribed dates during the first 6 months and also after 15 months. The iodine content of DFS and iodized salt stored under simulated household conditions was also measured in the first 3 months.

Results. After the color code was broken at the end of the study, it was found that the DFS and iodized salt

stored at Bhubaneswar, Delhi, and Hyderabad retained more or less the same initial iodine content (30–40 ppm) during the first 6 months, and the stability was not affected after 15 months. The proportion of salt samples having more than 30 ppm iodine was 100% in DFS and iodized salt throughout the study period. Daily opening and closing of salt pouches under simulated household conditions did not result in any iodine loss.

Conclusions. The DFS and iodized salt prepared by the dry-mixing method and stored at normal room conditions had excellent iodine stability for more than 1 year.

Key words: Double-fortified salt, iodine-deficiency disorders, iodized salt, iodine stability, iron-deficiency anemia, modified orthophosphoric acid method

Introduction

The control of micronutrient deficiencies by food fortification is one of the most significant developments in recent years. Probably no other technology available today offers such a wide scope to improve the health and nutritional status of people in the most cost-effective way [1, 2]. India has made rapid progress in this regard and has been successfully using low-cost technologies available for the production of iodized salt [3, 4] and iron-fortified salt [5, 6] in the country.

To tackle simultaneously the iodine-deficiency disorders and iron-deficiency anemia, iron- and iodine-fortified, double-fortified salt (DFS) was developed with refined common salt (100%); potassium iodate, KIO_3 (0.0067%); ferrous sulfate heptahydrate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.508%); and sodium hexametaphosphate (1%) to provide simultaneously about 40 µg of iodine and 1,000 µg of iron per gram of DFS [7]. Sodium hexametaphosphate (SHMP) is a permitted food additive [8] and is extensively used in the food industry. Scientific evaluation of the large-scale production and sensory acceptability [9], ultrastructure [10], biosafety of long-term

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consumption [11], impact, and efficacy [12-15] of DFS has been done.

When the iodine content of DFS is estimated by conventional iodometric titration [16] with the use of sulfuric acid, several problems may occur. There can be a wide variation in the iodine content of duplicate analyses of the same samples, and low iodine values have also been observed in freshly prepared DFS (8–31 ppm), although the expected iodine content was about 40 ppm. There was no consistency in the iodine content estimated over a period of time (10.1–22.6 ppm). In some batches, very high iodine content (50–70 ppm) was also obtained for the same sample of DFS. Lack of consistency in the iodine content of DFS created a lot of confusion regarding the stability of iodine in the multicenter study of DFS [17].

However, more detailed studies were carried out to exclude any undesirable element in DFS so as to ensure the primary objective of providing enough iodine and iron through DFS to achieve the goal of controlling iodine-deficiency disorders and iron-deficiency anemia. During this pursuit, a modified method using orthophosphoric acid was developed to solve the problems [18], and this method was used to test the iodine stability of DFS at two different laboratories (Delhi and Hyderabad) simultaneously on the same predetermined dates, ensuring uniform conditions of storage, duration of storage, and analysis. Loss of iodine, if any, was also measured on a weekly basis in salt pouches stored under simulated domestic conditions.

Materials and methods

Production

One-half metric ton each of DFS, iodized salt from refined salt (IRS), and iodized salt from ordinary common salt (IOS) were produced in the salt factory of M/S Prince International at Bhubaneswar, according to the dry-mixing process prescribed for iodized salt [3] and DFS [9]. M/S. Sahayamatha Salterns (P) Ltd., Tuticorin, supplied the refined salt, and M/S. Mohapatra Scientific Supply Syndicate, Bhubaneswar, supplied ordinary common salt, SHMP, ferrous sulfate, potassium iodate, low-density polyethylene (LDPE) pouches, and high-density polyethylene (HDPE) bags. Food-grade chemicals were used for the production of DFS and iodized salt. The pH of a 5% aqueous solution of SHMP was 6.0, and its purity determined by its P_2O_5 content was 68%. Both of these values were well within the prescribed limits for SHMP for use in DFS. The refined salt (99.5% NaCl) was white with an average crystal diameter of 100 μm in and contained 0.01% magnesium, 0.01% calcium, 0.05% sulfate, 0.14% moisture, and 0.01% insoluble residue. The ordinary common salt (96.0% NaCl) was off-white with an

average crystal diameter of 500 μm in and contained 0.10% magnesium, 0.18% calcium, 0.50% sulfate, 3.8% moisture, and 0.80% insoluble residue.

After estimation of the initial iron content of DFS and the initial iodine content of DFS, IRS, and IOS, the three salts were packed and sealed in color-coded 0.5-kg LDPE pouches (white, orange, or yellow) in the salt factory by the factory staff. Twenty-five LDPE pouches were further packed and sealed in double-lined HDPE bags of the same colors. The managing director of the salt factory gave the color code to ensure blinding. The key to the code was handed over to the director of the National Institute of Nutrition (NIN) in a sealed cover for safe custody and was broken at the end of the study. The investigators of the two laboratories were blinded to the type of salt until the color code was broken at the completion of the study.

Transportation

The LDPE salt pouches in HDPE bags, placed in closed containers protected from light, were transported from the factory by road and reached NIN, Hyderabad (17 HDPE bags per color \times 3 = 51 HDPE bags containing 1,275 LDPE pouches), and the laboratory of the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), New Delhi (17 HDPE bags per color \times 3 = 51 HDPE bags containing 1,275 LDPE pouches), in a week. The remaining HDPE bags (6 HDPE bags per color \times 3 = 18 HDPE bags containing 450 LDPE pouches) were sent to the Orissa Unit of the National Nutrition Monitoring Bureau (NNMB) at the Regional Medical Research Centre, Bhubaneswar, to assess the stability of iodine in a coastal environment.

Storage

The HDPE bags containing 25 LDPE salt pouches per HDPE bag were stored in rooms in Bhubaneswar, Hyderabad, and New Delhi. The average maximum and minimum temperatures were 40° and 23°C during February, March, April, and May (summer), 32° and 20°C during June, July, August, and September (rainy season), and 26° and 10°C during October, November, December, and January (winter). The average relative humidity was 18% in the summer, 90% in the rainy season, and 49% in the winter. The average rainfall was about 500 mm during the rainy season, 45 mm during the summer, and a trace in the winter.

Sampling and testing of fortified salts for iodine stability

Sampling of DFS and iodized salt and estimation of iodine were performed simultaneously at the NIN and ICCIDD laboratories on the same predetermined dates to ensure uniform conditions of storage, duration

of storage, and analysis. The investigators of the two laboratories did not know the type of salt until the color code was broken at the end of the study and were measuring the iodine content based on the color code of the LDPE pouch. Every month during the first 6 months on the predetermined date, one LDPE pouch per HDPE bag (from 25 LDPE pouches) was randomly picked up and the iodine content was estimated in each LDPE pouch by taking duplicate aliquots of each sample. On the predetermined date every month, the staff of the NNMB Orissa Unit dispatched randomly picked salt samples (LDPE pouches) to the NIN and ICCIDD laboratories, and the salt samples were analyzed simultaneously in the two laboratories. Loss of iodine, if any, under simulated household conditions was measured in a sub-sample of each category of fortified salt. For this purpose, six LDPE pouches of each category of salt (white, orange, or yellow) were drawn randomly, and after estimation of the initial iodine content, the salt pouches were closed with rubber bands and stored. Subsequent estimation of iodine in these salt pouches was performed on the 7th, 14th, and 21st days of every month during the first 3 months. These salt pouches were opened and closed daily, as salt is generally handled in the households.

Standardization of iodine estimation method in DFS and iodized salt

Following the conventional iodometric titration used for the estimation of iodine in iodized salt, 10 g of iodized salt was dissolved in 50 mL of distilled water, and 1 mL of 2 N sulfuric acid (H_2SO_4) was added followed by the addition of 5 mL of 10% potassium iodide (KI). The reaction mixture was kept in the dark for 10 minutes, and the iodine liberated was estimated by titration with 0.005 M sodium thiosulfate ($Na_2S_2O_3$) using a starch indicator near the end point of titration (table 1).

Kolthoff and Belcher have recommended the use of orthophosphoric acid (H_3PO_4) instead of H_2SO_4 for iodine estimation in the presence of iron to overcome any interference from iron [19]. According to Kolthoff and Belcher, iodine should be liberated only after sufficient iodide (KI) is present in the solution to minimize the loss of iodine by volatilization [20]. This is accomplished by adding iodide before the addition of acid. The pH of a solution of 10 g of salt in 50 mL of distilled water was acidic (2.0) for DFS and alkaline (7 to 8) for iodized salt. However, iodine is not stable at the acidic pH of DFS. We therefore used H_3PO_4 in the procedure and modified the method by adding 0.50 mL of 1% KI first to 10 g of DFS or iodized salt followed by 50 mL of distilled water and 5 mL of 4N H_3PO_4 , and titration was done with 0.005M $Na_2S_2O_3$ after keeping the solution in the dark for 10 minutes [18]. The modified method showed excellent agree-

TABLE 1. Methods used in the estimation of iodine in double-fortified salt (DFS) and iodized salt (IS)^a

Method	Acid used	Procedure
Conventional titration ^b	Sulfuric acid (H_2SO_4)	10 g DFS or IS + 50 mL distilled water + 1 mL 2N H_2SO_4 + 5 mL 10% KI. Keep in dark for 10 min and titrate with 0.005M $Na_2S_2O_3$.
Modified method ^c	Orthophosphoric acid (H_3PO_4)	10 g DFS or IS + 0.5 mL 1% KI + 50 mL distilled water + 5 mL 4N H_3PO_4 Keep in dark for 10 min and titrate with 0.005M $Na_2S_2O_3$.

a. Values based on reference salt & KIO_3 standard

b. Sullivan et al. [16].

c. Ranganathan et al. [18].

ment with the conventional titration method for the estimation of iodine ($r^2 = 0.9998$) both in the KIO_3 standard at different iodine levels and also in iodized salt from the factory or market [18]. Therefore, the NIN and ICCIDD laboratories employed the modified method for the estimation of iodine in DFS, IRS, and IOS throughout the study period, in order to ensure uniformity in methodology.

Quality control

In order to ensure the reliability of results, the NIN and ICCIDD laboratories strictly adhered to internal as well as external quality control measures for iodine estimation. For internal quality control, multiple analyses (20 times) of the iodine content of the KIO_3 standard (1 mg iodine/mL) in 10 g of plain noniodized salt were performed. The 95% confidence interval of mean iodine was calculated along with the operating control range (lower limit, mean - 2 SD; upper limit, mean + 2 SD) for preparing the quality control charts. Furthermore, the same procedure was adopted using a reference salt with a known level of iodine to which 1,000 ppm of iron and 100 mg of SHMP were added fresh before iodine estimation. Whenever the iodine content of DFS, IRS, or IOS was estimated on the predetermined dates in the two laboratories, the iodine content of reference salt (with known levels of iodine and iron) and the KIO_3 standard were also measured.

For external quality control, 10 samples each of DFS, IRS, and IOS drawn randomly at NIN were sent to the ICCIDD laboratory. The salt samples were measured in duplicate for iodine content simultaneously by the investigators from both of the centers using the same reagents as the ICCIDD laboratory.

Estimation of iron in DFS

Soon after production (before color coding in the factory) and at the end of the study (after the code was broken) the iron content of DFS was determined according to the method of Wong [21].

Statistical analysis

Analysis of variance was performed with the laboratories and duration of storage as independent variables and iodine values as the dependent variable. The percentage frequency distribution according to iodine content of the three categories of salt was also determined. Regression analysis and *t*-tests were performed wherever necessary.

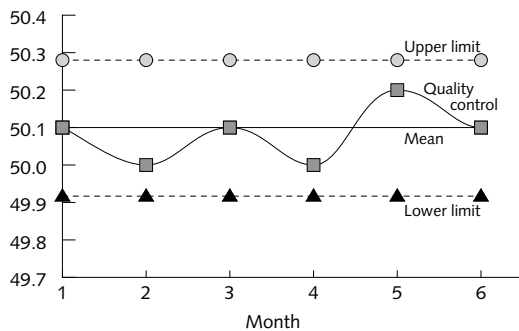


FIG. 1. Internal quality control chart of KIO₃ standard. Mean \pm 2 SD = 50.1 \pm 0.18 ppm; CV = 0.18%

Breaking of the code

Breaking the code for the fortified salts at the end of the study revealed that the orange pouches contained DFS, yellow pouches IRS, and white pouches IOS.

Results

Initial iodine content

The initial iodine content (mean \pm SD) was 40.3 \pm 3.8 ppm in DFS, 42.7 \pm 3.5 ppm in IRS, and 30.0 \pm 2.0 ppm in IOS.

Quality control

The result of the internal quality control of the KIO₃ standard showed that the operating range of iodine content for the KIO₃ standard was 49.92 to 50.28 ppm, with a coefficient of variation of 0.18% (fig. 1), and 45.16 to 45.64 ppm with a coefficient of variation of 0.26% for the reference salt (with known levels of iodine and iron). The day-to-day values of the reference salt (with known levels of iodine and iron) and the KIO₃ standard were well within these ranges throughout the study period at the two laboratories, indicating effective internal quality control.

The external quality control revealed good agreement between the duplicate values, within and between the laboratories, irrespective of DFS, IRS, or IOS. The iodine content (mean \pm SD) of DFS was 41.5 \pm 2.9 ppm

TABLE 2. Iodine content of fortified salts stored at Hyderabad, Delhi, and Bhubaneswar according to number of months of storage

Month	Mean \pm SD iodine content (ppm)					
	Double-fortified salt		Iodized refined salt		Iodized ordinary salt	
	NIN	ICCIDD	NIN	ICCIDD	NIN	ICCIDD
Hyderabad and Delhi (<i>n</i> = 17 samples per salt per laboratory for each measurement)						
1	40.1 \pm 4.3	40.1 \pm 4.3	44.3 \pm 4.0	44.5 \pm 6.0	30.7 \pm 2.7	33.6 \pm 4.2
2	42.5 \pm 4.7	42.0 \pm 4.2	43.8 \pm 4.9	45.5 \pm 4.0	32.4 \pm 1.9	33.9 \pm 2.1
3	42.0 \pm 3.6	39.3 \pm 1.8	43.1 \pm 3.3	43.0 \pm 3.3	31.9 \pm 1.2	31.2 \pm 1.5
4	42.0 \pm 3.6	39.3 \pm 1.8*	42.9 \pm 2.5	40.3 \pm 2.1	32.4 \pm 3.0	30.8 \pm 1.8*
5	41.4 \pm 3.3	40.4 \pm 1.6	41.4 \pm 3.0	38.2 \pm 1.8*	30.2 \pm 0.9	28.8 \pm 1.6*
6	40.2 \pm 2.1	39.6 \pm 1.8	41.6 \pm 2.3	37.6 \pm 2.4*	30.0 \pm 1.2	28.2 \pm 1.4*
Bhubaneswar (<i>n</i> = 6 samples per salt per laboratory for each measurement)						
1	40.2 \pm 1.4	43.5 \pm 3.0	41.2 \pm 1.1	42.3 \pm 1.5	33.6 \pm 1.0	34.0 \pm 0.8
2	40.9 \pm 1.3	41.6 \pm 1.0	41.4 \pm 2.1	41.3 \pm 3.4	32.8 \pm 1.4	31.9 \pm 1.4
3	44.4 \pm 3.3	40.2 \pm 0.7	42.5 \pm 5.6	38.5 \pm 2.2*	30.8 \pm 1.0	30.3 \pm 0.2
4	44.4 \pm 3.3	41.3 \pm 1.1	43.2 \pm 5.0	40.2 \pm 1.0*	32.0 \pm 0.3	31.4 \pm 0.8
5	40.7 \pm 0.6	39.8 \pm 0.9	44.0 \pm 0.6	40.0 \pm 1.6*	28.7 \pm 2.6	30.7 \pm 0.9*
6	40.7 \pm 0.9	40.4 \pm 0.8	40.5 \pm 1.1	40.0 \pm 0.8	29.8 \pm 0.8	29.5 \pm 0.8

NIN, National Institute of Nutrition; ICCIDD, International Council for Control of Iodine Deficiency Disorders

*Difference between laboratories significant at *p* < .05 (*t*-test).

for NIN and 42.5 ± 3.0 ppm for ICCIDD; the iodine content of IRS was 46.1 ± 2.8 ppm for NIN and 46.0 ± 3.1 ppm for ICCIDD; and the iodine content of IOS was 35.1 ± 2.9 ppm for NIN and 35.9 ± 2.8 ppm for ICCIDD. The intraclass correlation was close to unity ($\rho = 0.97$).

Iodine content of DFS, IRS, and IOS stored at Hyderabad and New Delhi

Regular monthly analysis of the salt samples carried out at the two laboratories showed that the mean iodine content of DFS as well as IRS was about 40 ppm, while that of IOS was 30 ppm (table 2).

The iodine levels of all three fortified salts during the study period were essentially the same as their initial levels. Analysis of variance showed that there was no difference in iodine content between the two laboratories and at different time points within the same laboratory. However, there were minor differences in iodine content between the laboratories at some points ($p < .05$), mainly in IOS and IRS (table 2). These differences could be attributed to batch-to-batch variations at the time of production. Since the frequency distributions of the iodine values were nearly identical, these differences are of no practical relevance. Furthermore, the percentage of salt samples having ≥ 30 ppm iodine was 100% in DFS, IRS, and IOS throughout the study period (mandatory level: 15 to 30 ppm).

Iodine content of DFS, IRS, and IOS stored at Bhubaneswar

The iodine contents of the three fortified salts stored at Bhubaneswar and tested at the NIN and ICCIDD laboratories are given in table 2. The mean iodine content of DFS and IRS was about 40 ppm, whereas that of IOS was 30 ppm throughout the 6-month period. No significant differences, in general, were observed in the iodine content.

TABLE 3. Iodine content of fortified salts after 15 months of storage

Salt	Mean \pm SD iodine content (ppm) ($n = 17$ samples per salt per laboratory)	
	NIN	ICCIDD
Double-fortified	31.9 ± 1.8	31.7 ± 2.2
Iodized refined	41.2 ± 0.9	42.9 ± 3.1
Iodized ordinary	29.8 ± 0.7	30.0 ± 1.5

NIN, National Institute of Nutrition; ICCIDD, International Council for Control of Iodine Deficiency Disorders

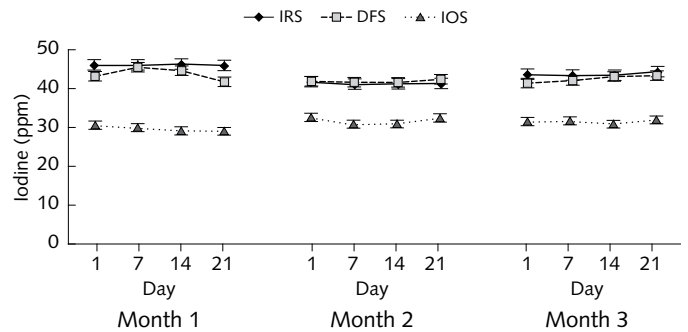


FIG. 2. Weekly variation of iodine content of fortified salts during the first 3 months. DFS, double-fortified salt; IRS, iodized refined salt; IOS, iodized ordinary salt

Iodine content of DFS, IRS, and IOS after 15 months

In view of the excellent iodine stability observed in the first 6 months, all of the salt samples were stored for a long time and the iodine content was measured in these samples after 15 months by the same protocol and sampling procedures followed during the first 6 months. The results revealed consistency in iodine stability even after 15 months (table 3); DFS and IOS had about 30 ppm iodine and IRS about 40 ppm iodine. The percentage of salt samples having ≥ 30 ppm iodine was 100% in DFS, IRS, and IOS even after 15 months (mandatory level: 15 to 30 ppm).

Variation of iodine content in the fortified salts stored under simulated household conditions

Weekly analysis of subsamples of DFS, IRS, and IOS stored under simulated household conditions during the first 3 months of the study period revealed no significant changes in iodine content; the mean iodine contents of DFS and IRS were about 40 ppm, and that of IOS was 30 ppm (fig. 2).

Overall iodine stability

The study revealed that the stability of iodine was consistently satisfactory in DFS, IRS, and IOS (fig. 3). All three types of fortified salt had ≥ 30 ppm iodine, even after 15 months.

Iron content of DFS

The mean iron content of DFS was $1,030 \pm 62$ ppm soon after production ($n = 40$) and $1,034 \pm 58$ ppm at the end of 6 months ($n = 40$), indicating satisfactory iron stability.

Discussion

This study has reconfirmed the large-scale production

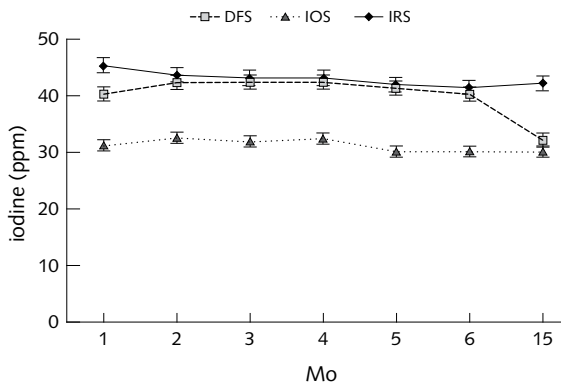


FIG. 3. Mean iodine content of fortified salts according to duration of storage. Mandatory iodine level, 15 to 30 ppm. DFS, double-fortified salt; IRS, iodized refined salt; IOS, iodized ordinary salt

of DFS and iodized salt by the dry-mixing method and transportation over long distances. The initial iodine content of IOS was less (30 ppm) than that of IRS and DFS (40 ppm), although the same amount of KIO_3 was used in all three salts. This difference in iodine levels could be attributed to the composition of common salts, especially the higher levels of moisture (3.8%) and magnesium (0.10%) in ordinary salt, as compared with the low levels of moisture (0.14%) and magnesium (0.01%) in refined salt. Earlier studies of iodized salt revealed a certain amount of iodine loss during the initial months, although KIO_3 was used as the source of iodine. This was attributed to the use of water in the spray-mixing process employed for the production of iodized salt [4, 22-25]. In contrast, no iodine loss was observed when the dry-mixing process was adopted, and excellent iodine stability was ensured during prolonged storage [3, 17, 22, 26]. The present study confirms these observations.

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The monthly testing of DFS and iodized salt revealed that DFS and iodized salt stored at Bhubaneswar, Hyderabad, and New Delhi had excellent iodine stability and the iodine content was always ≥ 30 ppm in 100% of the samples tested throughout the study period; the salt pouches stored and handled under simulated household conditions did not show any iodine loss in DFS and iodized salt; the modified method using H_3PO_4 is suitable not only for DFS but also for iodized salt; and the coastal environmental conditions at Bhubaneswar did not affect the stability of iodine in DFS or iodized salt, indicating that the poor iodine stability observed earlier in DFS at Bhubaneswar [17] could be due to the inherent problems in the method of iodine estimation followed at that time.

It can therefore be concluded that DFS prepared according to the NIN formula had excellent iodine stability even after 15 months. This study confirmed the usefulness of the modified method for estimation of iodine in iodized salt, whether the salt is derived from ordinary common salt or from refined common salt.

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