Application of Quantitative Salt Iodine Analysis Compared with the Standard Method

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Abstract

Laboratory investigation of 50 iodated salt samples (from producers, households, markets etc) were studied at the Research Nuclear Medicine Building, Siriraj Hospital. Two methods for the determination of iodine in salt are herein described. The standard method as recommended by The Programme Against Micronutrient Malnutrition (PAMM) / The Micronutrient Initiative (MI) / The International Council for Control of Iodine Deficiency Disorders (ICCIDD) was the iodometric titration method. The starch-KI salt iodine quantitative method was developed in our laboratory for validation purposes. This method is high in precision, accuracy, sensitivity as well as specificity. The coefficient of variation (%CV) for intra and inter assay was below 10. Iodine contents as low as 10 ppm, could be detected. The proposed starch-KI method offered some advantages : e.g. not complicated, easier to learn and easier to perform competently, could be applied for spot qualitative test and readily performed outside the laboratory. The results obtained by the starch-KI method correlated well with the standard method ($y = 0.98x - 3.22$, $r = 0.99$).

Key word : Iodated Salt, Iodometric Titration, Starch-KI Method, Spot Qualitative Test

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Reliable, accurate and timely evaluation of the iodine content of salt is key to verifying progress towards the global golds of eliminating Iodine Deficiency Disorders (IDD). There are a number of methods for testing the iodine content of salt, ranging from qualitative methods which are useful in field settings, to more quantitative methods performed in laboratories for validation purposes. Dif-

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ferent salt iodine test methods need to be used depending on the form of iodine (iodate or iodide) used in fortification. From our past experience in some of the original work on iodine experiments[1], it may be concluded that KIO_3 is the compound of choice for iodine fortification under tropical conditions. The standard method for measuring iodine in iodated salt as recommended by PAMM[2] is the iodometric titration method. Titration is recommended to determine the exact concentration of iodine in salt at various levels of the distribution system where accurate testing is required. However, this testing is too time consuming and expensive for routine monitoring purposes throughout the country.

We have developed a quantitative method performed in our laboratory. This "starch-KI" salt iodine quantitative method is modified from Dustin and Ecoffey[3]. The principle of the reaction for quantitate iodine in iodated salt is based on the oxidation-reduction of KIO_3. KIO_3 in salt is reduced by KI and HCl to liberate free iodine. The free iodine will react with a starch solution and produce a blue color. The intensity of the blue color is proportional to the iodine content in salt and measured by a spectrophotometer.

$$KIO_3 + 5KI + 6HCl \longrightarrow 3I_2 + 3H_2O + 6KCl$$
$$I_2 + \text{starch} \longrightarrow \text{blue color}$$

MATERIAL AND METHOD

It was practical to collect 30-50 samples of iodated salt from producers, households, markets, etc and analyse their iodine content in our laboratory at Siriraj Hospital using the starch-KI method. The iodometric titration method was used as the gold standard method for determination of iodine in salt.

Starch-KI method

The apparatus involved a spectrophotometer (UV/Visible Spectrophotometer, Philips Scientific) and solutions described below. Glass-distilled deionized water was used for preparation of reagents and dilution procedures. Water had to be free of iodine and other contaminants.

The reagents for the starch-KI method were hydrochloric acid solution (2.7 mol/l HCl) and starch-KI solution (1% starch or cassava flour, 3% potassium iodide and 0.4% sodium azide). For standard iodine solution, the stock iodine standard solution (A) was prepared by dissolving 16.86 mg of potassium iodate (KIO_3) in a 100 ml volumetric flask with deionized water. This solution was equivalent to 100 ppm. For solution B, diluted from solution A 10 fold with deionized water, the iodine concentration was 10 ppm. The working standards, ranging from 0, 0.1, 0.3, 0.5, 0.7, 1.0 ppm iodine were prepared by diluting 0, 0.1, 0.3, 0.5, 0.7 and 1.0 ml of solution B, respectively, with deionized water to 10 ml. The preparation of unknown or salt sample, 1g of salt was dissolved in 80 ml deionized water, then the salt solution was filtered by Whatman No 3 filter paper, and the volume was made to 100 ml with deionized water.

The procedure involved the following steps. Pipette 3 ml of each working standard and salt samples in appropiate test tubes. 150 µl of HCl and 150 µl starch-KI solution were added, mixed, and kept in the dark for 15 minutes. The absorbance at 500 nm was read by a spectrophotometer (UV/Visible, Philips). Optical density at 500 nm (OD_{500}) was plotted against the iodine concentration for each standard and values for the salt samples were read from the standard curve.

Iodometric titration method

Details on the materials and procedure of the iodometric titration method were reported in 1995[2]. Briefly, the reagents are sodium thiosulfate solution (0.005 mol/l $Na_2S_2O_3$), sulfuric acid (1 mol/l H_2SO_4), starch indicator solution, and 10 per cent potassium iodide (KI).

The procedure for unknown sample, 10 g of salt sample was dissolved with 50 ml water in an Erlenmeyer flask, 1 mol/l H_2SO_4 1 ml and 10 per cent KI 5 ml were added, the solution should turn yellow if iodine is present. A stopper was put on the flask and placed in a dark drawer for 10 minutes. The burette was rinsed and filled with 0.005 mol/l sodium thiosulfate. The flask was removed from the drawer, and some sodium thiosulfate from the titration burette was added until the solution turned pale yellow. 2 ml of starch indicator solution was added, the solution should turn dark purple, and titrating was continued until the solution became pink, and finally colorless. The level of thiosulfate in the burette was recorded and converted to parts per million (ppm) of iodine in salt[2].

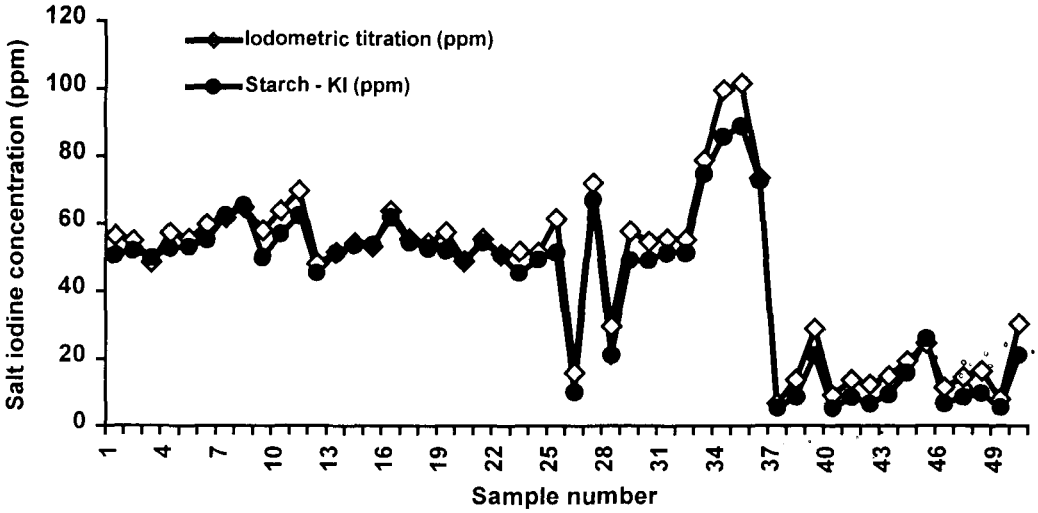

Fig. 1. Comparison of the starch-KI method and iodometric titration method (n=50).

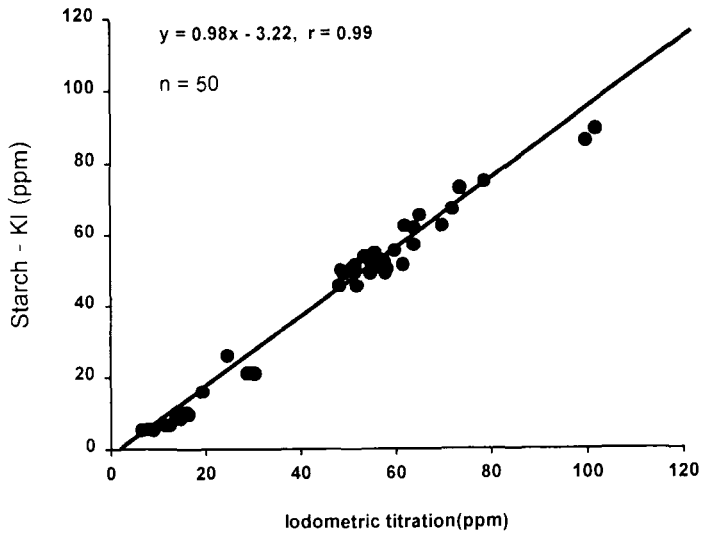

Fig. 2. Relationship between salt iodine concentrations determined by starch-KI and iodometric titration method.

Data analysis and statistics were based on the Pearson Correlation[4] and the Intra-Class Correlation or Agreement Index[5] using Microsoft Excel Version 7 software.

The external quality controls : One blind sample from the Centres for Disease Control and Prevention (CDC) in Atlanta, USA was sent regularly to the laboratory, usually four times a year.

Table 1. Correlation of salt iodine concentration obtained by iodometric titration method (x) and starch-KI method (y).

Method of analysis	Salt iodine range (ppm)	Mean iodine (ppm)	No. of samples	Regression equation	Correlation coefficient (r)	Intra-class Correlation Coefficient (ICC)
Iodometric titration (x)	Min : 6.6 Max : 101.8	46.6	50	y=0.98x-3.22	0.990 (p<0.0001)	0.987
Starch -KI (y)	Min : 5.3 Max : 89.1	42.4	50			

Table 2. Shows the analytical recovery of starch-KI method.

Iodine (ppm)			
Added	Observed	Measured*	% Recovery**
None	50.8		
5	55.6	4.8	96
10	60.9	10.1	101
20	70.1	19.3	96.5
Average			97.8

* Measured = Observed value corrected for value obtained with no iodine added

** % Recovery = (Measured ppm/added ppm) x 100

The salt samples were analysed for iodine content by iodometric titration method and starch-KI method.

RESULTS

Discrepancy between methods

Fig. 1 compares the salt iodine concentration for the samples in each method. Salt iodine concentration was plotted on a linear scale, with results of the two methods used to rank samples in parallel. Fig. 2 illustrates a direct correlation between the salt iodine concentration by starch-KI (y) and iodometric titration method (x). A regression equation of y = 0.98x –3.22, yielded the correlation of 0.99 which was highly significant for this relationship (p<0.0001). As Table 1 shows the mean salt iodine concentration measured by iodometric titration and starch-KI method were 46.6 and 42.4 ppm respectively. The Intra-Class Correlation (ICC) or Agreement Index value of salt iodine concentration obtained from the two methods was 0.987.

Assay performance

The assay performance of the starch-KI method is

A. Recovery : The mean (±SD) analytical recovery that was obtained in starch-KI method, after the addition of KIO_3, was 98 (±2.4) as shown in Table 2.

B. Sensitivity : The sensitivity of starch-KI method was calculated as the concentration corresponding to 2 standard deviations above the mean of the dose response variable (OD_{500}) measured in replicates of the unfortified salt as shown below.

Mean OD_{500} = 0.0305, SD = 0.0014, n = 30, % CV = 4.6.

The mean of OD_{500} + 2SD = 0.0305 + 2 (0.0014) = 0.0333.

The value calculated above (0.0333) was then read off the standard curve (Fig. 3). The concentration corresponding to the interest-point on the curve was taken to be the sensitivity, in this case it was 0.1 ppm in solution

$$= \frac{0.1 \times 10^2 \times 10^6}{10^6} = 10 \text{ ppm in salt}$$

C. Precision : As shown in Table 3, for intra assay, the CV for the assay of the control salt with the mean iodine level of 16.7 ppm in a total of 15 replicates was 5.3. For two controls with mean iodine concentration of 50.0 and 100.0 ppm, the CV was 2.7 (n=15) and 4.9 per cent (n=15), respectively.

The between-assay precision for the assay of three controls salt is shown in Table 4. The CV for the three controls salt, low medium and high level was 5.1 per cent, 6.3 per cent and 3.6 per cent, respectively.

The external quality controls for the assay of iodated salt obtained from CDC in Atlanta, USA

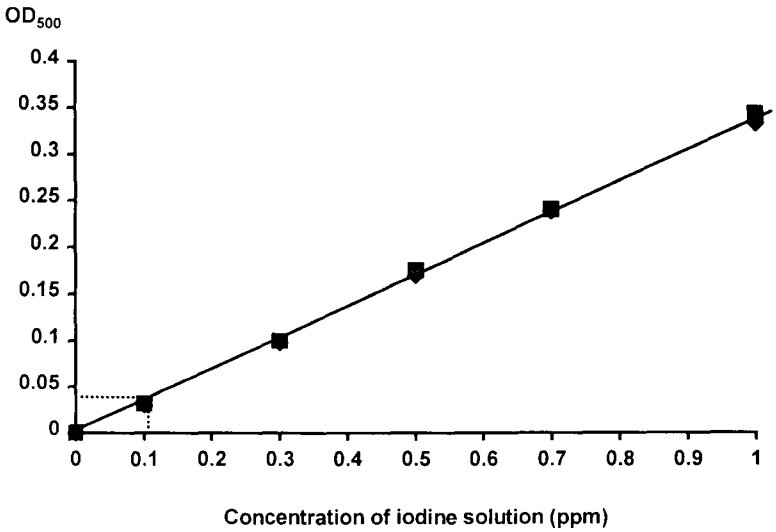

Fig. 3. The standard curve of the starch-KI method.

Table 3. Precision assays : intra assay of iodine determination in salt by starch-KI mehod.

Iodine level (ppm)	Mean iodine content (ppm)	Standard deviation (ppm)	No. of samples (n)	Coefficient of variation (%CV)
16.7	15.0	0.8	15	5.3
50.0	51.8	1.4	15	2.7
100.0	101.6	5.0	15	4.9

Table 4. Precision assays : inter assay of iodine determination in salt by starch-KI method.

Iodine level (ppm)	Mean iodine content (ppm)	Standard deviation (ppm)	No. of samples (n)	Coefficient of variation (%CV)
Low	15.6	0.6	10	5.1
Medium	52.4	3.3	10	6.3
High	98.0	6.3	10	3.6

are shown in Table 5. The CV for the assay with a mean salt iodine concentration of 77.5 ppm, in a total of 3 batches assay of iodometric titration (n=12), was 3.6 per cent. In the starch-KI method, the between-assay CV for iodated salt with a mean of 76.2 ppm was 5.6 per cent. The target range of the external quality controls in each batch was 70-85 ppm.

DISCUSSION

Comparison of the developed starch-KI method with the standard iodometric titration method is one of the effective tools to evaluate the technique. The results show a highly satisfactory correlation of salt iodine concentration between the starch-KI and iodometric titration method (r= 0.990). For ICC suggests not only good correlation between the two methods but also close value (ICC=0.987). From our studies, we can conclude that the starch-KI method is high in precision, accuracy, sensitivity, and should be applied for spot qualitative test which is easily performed outside the laboratory. This spot test method is rapid, simple, low cost and is easily applicable in the

Table 5. External quality controls for iodated salt analyzed by iodometric titration and starch-KI method.

Batch No.	Mean (SD) salt iodine, ppm (n = 12)		Control iodated salt target range, ppm
	Iodometric	Starch - KI	
1	78.9 (0.6)	74.0 (3.6)	
2	73.6 (1.6)	72.5 (2.5)	70-85
3	80.0 (2.5)	82.2 (2.6)	

field. Individuals without specific chemistry training can easily verify whether a salt sample has been iodized. Such monitorings will be particularly needed in the situation where products from medium to large scale salt producers need to be processed as evidence or as part of their quality control. They will also be helpful for government agencies who take responsibilities in regulating the iodine content in salt obtained from various sources including producers, households, markets, warehouses and importers.

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การประยุกต์การวิเคราะห์หาปริมาณไอโอดีนในเกลือเทียบกับวิธีมาตรฐาน

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ได้ทำการเปรียบเทียบระดับไอโอดีนในตัวอย่างเกลือไอโอเดท จำนวน 50 ตัวอย่าง ซึ่งได้จากแหล่งผลิตเกลือ, ในครัวเรือน, ร้านค้าในตลาด โดยวิธีมาตรฐาน iodometric titration กับ วิธีดัดแปลง starch–KI ซึ่งเป็นวิธีที่มีความเที่ยงตรง ความแม่นยำ ความไว และความจำเพาะสูง ค่าสัมประสิทธิ์แห่งการกระจายของการวิเคราะห์ในการทดลองชุดเดียวกัน และต่างชุดการทดลอง หรือต่างวันกันน้อยกว่า 10 เปอร์เซ็นต์ สามารถตรวจหาไอโอดีนในปริมาณต่ำได้ถึง 10 ส่วนในล้านส่วน เป็นวิธีที่ไม่ยุ่งยาก ทำได้ง่าย สามารถประยุกต์ทำเป็นวิธีการตรวจภาคสนามได้ผลดี ผลการทดลองที่ได้จากวิธี starch–KI จะมีความสัมพันธ์กับวิธีมาตรฐาน โดยมีสมการเป็น y = 0.98 x – 3.22 และค่าสัมประสิทธิ์ของความสัมพันธ์เป็น 0.99

คำสำคัญ : เกลือไอโอเดท, วิธีไตเตรทแบบไอโอโดเมตริก, วิธีน้ำแป้ง–โปตัสเซียมไอโอไดด์, วิธีทดสอบภาคสนาม

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