Development of Sample Treatment Method for Flow Injection Determination of Iodine in Eggs: a Comparison Study

Todsaporn Srivorakul,*,** Pakorn Varanusupakul,*,**† Waleed Alahmad,*,**†

*Chemical Approaches for Food Applications Research Group, Faculty of Science, Chulalongkorn University, 254 Phayathai Rd., Pathumwan, Bangkok 10330, Thailand.
**Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Rd., Pathumwan, Bangkok 10330, Thailand.

†To whom correspondence should be addressed.
E-mail addresses: pakorn.v@chula.ac.th (P. Varanusupakul), waleed.a@chula.ac.th (W. Alahmad).
Abstract

A simple treatment method was proposed for the determination of iodine in eggs, followed by a flow injection spectrophotometry based on the catalytic effect of iodine on the reduction reaction of Ce(IV) with As(III). The egg matrix was removed based on protein precipitation principles. Several protein precipitation methods were investigated. The treatment using trichloroacetic acid satisfactorily removed most of the egg matrix components. A colorless solution and a good signal were achieved. The method provided more reliable results compared to the conventional alkali dry ashing.

Keywords: iodine; egg; protein precipitation; trichloroacetic acid; flow injection analysis; Sandell-Kolthoff
Introduction

Iodine is known as an important nutrient element in humans that plays an important role in brain development, metabolic processes, and several organ functions, especially in children and pregnant women. The daily intake amounts of iodine recommended by the World Health Organization (WHO) are 150 µg per day for adults and 250 µg per day for pregnant and breastfeeding women. An iodine-deficient diet could lead to iodine deficiency disorders such as goiter, cretinism, and hypothyroidism. Iodine-rich foods, such as seafood, seaweed, and plants grown in iodine-rich soil are common sources of an iodine diet. To ensure the prevention of iodine deficiency disorders problems, iodine-fortified food products, such as iodized table salt, iodine enriched dairy products, and eggs are available on the markets. Iodine-enriched eggs are of particular interest because eggs are a common food for all generations and are easily accessible to many people in all areas. Charoensiriwatana et al. reported that iodine-enriched eggs could solve the iodine deficiency endemic for remote areas in Thailand. Typical iodine-enriched eggs are produced by feeding the hens with iodine supplements. Recently, Suvanprakorn et al. invented a method for the iodine fortification of eggs based on iontophoresis. In order to control the quality and provide information about the iodine content in iodine-fortified eggs, a method for the determination of the iodine content in egg is required.

There are several analytical methods for the determination of iodine, such as inductively coupled plasma-mass spectrometry, gas chromatography-mass spectrometry, and ion chromatography. Although these analytical techniques have high sensitivity and precision, the analysis is time-consuming, the instruments are expensive and the requirement to trained operators. Alternatively, colorimetric methods are low cost and simply measured by a spectrophotometer. The Sandell-Kolthoff method is the most sensitive and simple colorimetric method for the determination of iodine, where the yellow color of Ce(VI) is reduced by As(III) to the colorless Ce(III) catalyzed by a quantity of iodine. Recently, the method has been modified for being used in a flow injection system. Nevertheless, the determination of
iodine in food samples usually requires sample preparation steps to remove unwanted food matrices and isolate iodine into an appropriate solution prior to determination. Several sample preparation methods have been utilized for the determination of iodine in food and biological samples, such as Schöninger combustion\textsuperscript{19}, alkali dry ashing\textsuperscript{14,20-22}, and alkali extraction using tetramethyl ammonium hydroxide (TMAH)\textsuperscript{7,9,10}. Dusova et al.\textsuperscript{23} used the spectrophotometric Sandell-Kolthoff method after incineration in alkaline solution for determination of iodine content in eggs. Furthermore, Todorov et al.\textsuperscript{24} also used the spectrophotometric Sandell-Kolthoff method for determination of iodine in eggs after digestion the eggs using a ternary acid mixture of nitric acid, perchloric acid, and sulfuric acid. However, these methods consist of several steps, use high temperature or harsh conditions and require long preparation times. For these reasons, the development of a simple, fast, inexpensive, and environment-friendly sample preparation method for the determination of iodine in food is highly needed in the analytical community.

Since the matrix of eggs is mainly composed of proteins, lipoproteins, and lipids\textsuperscript{25}; methods that can remove proteins, therefore, require particular attention. Proteins are chains of amino acids containing hydrophilic and hydrophobic patches on the surface, making them varied in solubility and dispersed in aqueous solution as an emulsion. Proteins can be removed by lowering the solubility or changing conformation (denaturation) so that they can be aggregated and finally precipitated out of the solution\textsuperscript{26}. Several methods were used for this purpose such as chloroform-methanol mixture\textsuperscript{27}, trichloroacetic acid as common method\textsuperscript{28}, and trichloroacetic acid/acetone for samples with high levels of lipids\textsuperscript{29}. To date, no one of these sample treatments was used for protein precipitation in eggs samples coupled with the spectrophotometric Sandell-Kolthoff method.
In this work, we tried to investigate and develop a simple method for protein precipitation in order to remove the egg matrix prior to determination of iodine using the flow based Sandell-Kolthoff colorimetric method. Four different methods; namely, isoelectric point (pH adjustment), salting out, protein precipitation with sodium dodecylsulfate (SDS), and protein precipitation with trichloroacetic acid (TCA) were examined and evaluated. The method that gave the best analytical performance was successfully applied to iodine detections in different eggs samples and compared with the standard Alkali dry ashing method.

Materials and methods

Materials

Iodine solution was prepared by dissolving potassium iodate (Carlo-Erba, France) in deionized water (Millipore, USA). Trichloroacetic acid (TCA) was purchased from Carlo-Erba, France. Sodium dodecylsulfate (SDS) was purchased from Sigma-Aldrich, USA. Ceric sulfate and arsenious oxide (A&M, USA) were used in the Sandell-Kolthoff method. Hydrochloric acid, sulfuric acid, acetic acid, and sodium hydroxide were purchased from Merck, USA. Ammonium sulfate and zinc sulfate heptahydrate (Carlo-Erba, France) and potassium carbonate (Ajax) were used for the alkali ashing method. Egg samples were purchased from a local supermarket and stored in a refrigerator. Five eggs were cracked and only yolk and white egg were homogenized and used as a composited egg sample.

Egg sample treatment processes

A 0.5 g sample of homogenized egg was treated with four different processes, namely, pH adjustment, salting out, protein precipitation with SDS, and protein precipitation with TCA. In the pH adjustment process, solutions with different concentrations of hydrochloric acid and sodium hydroxide giving a pH ranging from 1-14 were used. In the salting out process,
solutions of saturated sodium chloride and sodium sulfate with different volumes ranging from 0.5 – 5 mL were studied. In the protein precipitation with SDS, the egg sample was first treated with a mixture solution of 1% SDS and 0.2 M sodium hydroxide at the ratio of 1:1 with different volumes of 0.4, 0.8, 1.2, 1.6 and 2 mL, and then a 3 M acetate buffer was added to the solution at the ratio of 3:4. In the protein precipitation with TCA, the egg sample was first treated with 1 g mL\(^{-1}\) of TCA with different volumes of 100, 200, 400, 800 µL. The final solution of 10 mL was centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatant was filtered with a 0.45-µm syringe filter prior to further analysis by the flow based Sandell-Kolthoff colorimetric method.

**Alkali dry ashing**

The performance of the developed method for treatment of egg samples was compared with the alkali dry ashing method. A 0.5 g of homogenized egg was mixed with 0.5 mL of 10% w/v zinc sulfate and 0.5 mL of 30% w/v potassium carbonate in a porcelain crucible. The sample was gently heated to dryness. Then, the sample was incinerated in a furnace at 550 °C for 1 h. Another 0.5 mL of 10% w/v zinc sulfate was added to the sample and incinerated again for 2 h. The white residue was dissolved in 10 mL deionized water. The solution was centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatant was filtered with a 0.45-µm syringe filter prior to further analysis by the flow based Sandell-Kolthoff colorimetric method.

**Flow based Sandell-Kolthoff colorimetric method for determination of iodine**

The Sandell-Kolthoff colorimetric method is one of the most sensitive and simple method for determination of iodine, where the yellow color of Ce(VI) is reduced by As(III) to the colorless Ce(III) catalyzed by a quantity of iodine\(^{16}\). The flow-based Sandell-Kolthoff colorimetric system (schematically depicted in Fig. 1) was adopted and modified from
Nacapricha et al.\textsuperscript{18} for the determination of iodine. The fiber optic spectrophotometer with a z-flow cell was obtained from Ocean Optics (Dunedin, FL, USA). A solution of 0.25 mL was injected into a continuous flowing stream of a water carrier. The sample zone was mixed with 0.1 M As (III) and 0.008 M Ce(IV) and carried to the reaction coil and to the z-flow cell successively for detection at 420 nm.

**Results and discussion**

*Physical observation of treated egg samples*

The physical observation of egg samples after treatment with various treatment processes is summarized in Fig. 2. Egg samples after treatment with acidic and basic solutions (Fig. 2a) and the salting out process using saturated sodium sulfate and saturated sodium chloride (Fig. 2b) showed that the egg matrices were not satisfactorily removed. The samples could not be filtered through a 0.45-µm syringe filter, giving yellowish turbid solutions, so that they could not proceed to the determination step. This is perhaps due to the high amount of lipids (fats) in yolk. On the other hand, egg samples after treatment with SDS (Fig. 2c) and TCA (Fig. 2d) showed that the egg matrices were well sedimented. The samples were filterable to give colorless solutions, so that they can proceed to the determination step.

*Protein precipitation with SDS and TCA*

Egg samples were treated with SDS and TCA proceeded to the determination of iodine by the flow-based Sandell-Kolthoff colorimetric method. The signals of iodine obtained from the egg samples treated with SDS and TCA are summarized in Fig. 3a and Fig. 3b, respectively. The signals obtained from blank deionized water (1), iodine solution (2), treated deionized water (3) and treated iodine solution (4) were comparable, showing that both SDS and TCA did not significantly distort the signals. However, the signals obtained from egg samples and iodine spiked egg samples treated with SDS (Fig. 3a5 and Fig. 3a6, respectively) were slightly
deformed compared to those treated with TCA (Fig. 3b5 and Fig. 3b6, respectively). At present, the explanation of the source of this phenomenon may not be as accurate as possible, but it is suspected that one of the reasons belongs to the eggs matrix and thus SDS could not remove the entire eggs matrix effectively. Therefore, TCA was chosen for the egg sample treatment prior to the determination of iodine.

**Optimization of experimental parameters of TCA for egg matrix removal**

In order to obtain the highest sensitivity for iodine determination, the parameters that affected the protein precipitation by TCA such as the amounts of TCA, the incubation time, and the incubation temperature were examined.

Various volumes of 1 g mL\(^{-1}\) TCA of 100-800 μL were added to 0.5 g of homogenized egg samples spiked with iodine (500 μg/L KIO₃) and the final volume was made up to 10 mL with deionized water. The Fig 4 shows the recoveries of iodine spiked egg samples after adding a different volume of TCA. The results showed that the highest %recovery was obtained at additional volume of 400 μL of 1 g mL\(^{-1}\) TCA due to the effective removal of egg matrices. Herein, the volume 400 μL was chosen for further studies.

On the other hand; at low temperature, the kinetic energy of protein molecules might be decreased so that the protein molecule may be aggregated better than at room temperature. From this point, the incubation temperatures were investigated by adding 400 μL of g mL\(^{-1}\) of TCA to the egg samples and incubated at room temperature (25 °C) and 4 °C for 15 min. The results did not show a significant different in %recovery (99% and 97%, respectively) between these two temperatures. Therefore, the incubation at room temperature was chosen for next experiment.

Furthermore, the incubation time of egg matrix with TCA was studied because the matrix in the egg may take time to aggregate and precipitate. An aliquot of 400 μL of TCA (1 g mL\(^{-1}\)) was added to the egg sample and incubated at room temperature between 0 - 15 min. As
shown in Fig. 5, there was not a significant difference between % recoveries obtained at these different incubation times. Thus, this parameter is not critical and the egg samples were taken to centrifuge step directly after adding TCA.

Analytical features

The analytical merits of the developed method for protein precipitation before iodine determination in eggs were investigated. Under the optimal conditions, the method detection limit (MDL) was 0.58 µg iodine per g of egg while the method quantitation limit (MQL) was 0.94 µg iodine per g of egg. The % Recovery was in the range of 84-109% and % RSD was less than 6% for six replicate measurements of spiked egg samples at 500 µg L⁻¹.

Determination of iodine in egg samples

To evaluate the feasibility of the development TCA method for practical analysis, additional recovery experiment was conducted using egg sample spiked with 10 µg I. The content of iodine in egg samples treated with TCA was determined and compared to those treated with the conventional alkali dry ashing method. The results showed that there was a quite significant difference between the obtained recoveries from TCA and alkali dry ashing. The average recoveries of spiked iodine egg samples treated with the alkali dry ashing method were 48% with % relative standard deviation more than 10% while the average recoveries of spiked iodine egg samples treated with TCA were 102% with % relative standard deviation less than 2%.

To figure out the reason behind the low obtained recoveries from the conventional alkali dry ashing method, additional experiment was conducted. The ashing process was examined whether there was any loss of iodine during the ashing process by determining the
signals of standard iodine solution (potassium iodate) with and without the treatment by the alkali dry ashing method. The signals of iodine were not significantly different (Data not shown), verifying that there was no significant loss of iodine during the ashing process. Therefore, the egg matrices might have affected the ashing efficiency resulting in that iodine might not be liberated from the egg matrices effectively.

Moreover, the amounts of iodine obtained from different egg samples treated with both methods were compared and summarized in Table 1. Egg samples treated with TCA yielded higher amount of iodine compared to those treated with alkali dry ashing method (P<0.05 one-tailed t-test within the sample), especially when egg samples contain high level of iodine, such as iodine-enriched eggs. Apparently, the poor recovery for alkali dry ashing could have a significant effect on the amount of iodine obtained from egg samples, especially at high iodine concentration.

**Conclusion**

We have successfully developed a simple method for removing egg matrices prior to determine iodine in eggs the Sandell-Kolthoff colorimetric method based on the precipitation of protein by trichloroacetic acid. The conventional alkali dry ashing is affected by the egg matrices in liberating iodine and did not give an accurate result. Trichloroacetic acid has been proved to remove egg matrices effectively and provide reliable results. The detection limit of the developed method was 0.58 μg of iodine per g of egg. Good recoveries (84-109%) and acceptable RSD% (less than 6%) were obtained by our developed method. The treatment process using TCA is simple, consuming less chemicals and time, and more reliable than the conventional alkali dry ashing method. As far as we could ascertain, this is the first report using
trichloroacetic acid for egg treatment in order to determine iodine using the Sandell-Kolthoff method.

**Conflicts of interest**

There are no conflicts to declare.

**Acknowledgments**

This work was supported by Ratchadapisek Sompoch Endowment Fund, Chulalongkorn University (CU-59-013-FW). Waleed Alahmad would like to acknowledge Rachadapisek Sompot Fund for Postdoctoral Fellowship, Chulalongkorn University.
References

1. World Health Organization, UNCsF, International Council for Control of Iodine Deficiency Disorders, “Assessment of Iodine Deficiency Disorders and Monitoring their Elimination: A Guide for Programme Managers”, 2007, 3rd edn. World Health Organization, Geneva.

2. S. Sinawat, “Iodine Deficiency Disorders in Thailand. In: Victor RP, Gerard NB, Ronald W (eds) Comprehensive Handbook of Iodine”, 2009, Academic Press, San Diego, pp 1221-1226.

3. M. Haldimann, A. Alt, A. Blanc, and K. Blondeau, J. Food Compost. Anal., 2005, 18, 461.

4. W. Charoensiriwatana, P. Srijantr, P. Teeyapant, and J. Wongvilairattana, Nutr. J., 2010, 9, 68.

5. M.B. Zimmermann, “Iodine Deficiency Disorders and Their Correction Using Iodized Salt and/or Iodine Supplements. In: Iodine Chemistry and Applications”, 2014, John Wiley & Sons, Inc, pp 421-431. doi:10.1002/9781118909911.ch22

6. P. Suwanprakorn, L. Tanasugarn, A. Limahksohn, S. Sinawat, and P. Vuthipongse, United States Patent, 2002, 6410060.

7. P. A. Fecher, I. Goldmann, and A. Nagengast, J. Anal. At. Spectrom., 1998, 13, 977.

8. Limchoowong, N., P. Sricharoen, S. Techawongstien, S. Kongsri, and S. Chanthai, J. Braz. Chem. Soc., 2017, 28, 540.

9. G. Radlinger, and K.G. Heumann, Anal. Chem., 1998, 70, 2221.

10. T.I. Todorov, and P.J. Gray, Food Addit. Contam. Part A., 2016, 33, 282.

11. T.I. Todorov, T. Smith, A. Abdalla, S. Mapulanga, P. Holmes, M. Hamilton, T. Lewis, and M. McDonald, Food Anal. Methods, 2018, doi:10.1007/s12161-018-1301-3

12. P. Das, M. Gupta, A. Jain, and K.K. Verma, J. Chromatogr. A., 2004, 1023, 33.
13. H. Jing, L. Li-Na, M. Shi-Fen, C. Ya-Qi, and L. Yi-Qiang, Chin. J. Anal. Chem., 2008, 36, 187.
14. W. Alahmad, N. Tungkijansina, T. Kanetac, P. Varanusupakul, Talanta, 2018, 190, 78.
15. R.E.D. Moxon, and E. J. Dixon, Analyst, 1980, 105, 344.
16. E.B. Sandell, and I.M. Kolthoff, J. Am. Chem. Soc., 1943, 56, 1426.
17. N. Choengchan, K. Lukkanakul, N. Ratanawimarnwong, W. Waiyawat, P. Wilairat, and D. Nacapricha, Anal. Chim. Acta., 2003, 499, 115.
18. D. Nacapricha, S. Muangkaew, N. Ratanawimarnwong, J. Shiowatana, and K. Grudpan, Analyst, 2001, 126, 121.
19. P. Schramel, and S. Hasse, Mikrochim. Acta, 1994, 116, 205.
20. S. Andersson, and U. Forsman, J. Chromatogr. B Biomed. Sci. Appl., 1997, 692, 53.
21. O.P. Foss, L. V. Hankes, and D. D. Van Slyke, Clin. Chim. Acta, 1960, 5, 301.
22. G. Knapp, B. Maichin, P. Fecher, S. Hasse, and P. Schramel, Fresenius J. Anal. Chem., 1998, 362, 508.
23. H. Dušová, J. Trávníček, Z. Peksa, D. Falta, V. Pálka, Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2012, 6, 75.
24. T. I. Todorov, T. Smith, A. Abdalla, S. Mapulanga, P. Holmes, M. Hamilton, T. Lewis, M. McDonald, Food. Anal. Methods, 2018, 11, 3211.
25. M. Anton, “Composition and Structure of Hen Egg Yolk. In: Huopalahti R, López-Fandiño R, Anton M, Schade R (eds) Bioactive Egg Compounds” 2007, Springer-Verlag Berlin Heidelberg, pp 1-6.
26. J.P.D. Goldring, Methods in Molecular Biology, 2019, 1855, 41.
27. D. Wessel, U.-I. Flügge, Anal. Biochem., 1984, 138, 141.
28. U. Arnold, R. Ulbrich-Hofmann, Anal. Biochem. 1999, 271, 197.
29. R. Hao, C. Adoligbe, B. Jiang, X. Zhao, L. Gui, K. Qu, S. Wu, L. Zan, *PLoS One*, 2015, 10, doi: 10.1371/journal.pone.0124723.
List of Tables

Table 1 Amounts of iodine in egg samples after treatment by TCA and alkali dry ashing method

| Egg sample              | Iodine concentration (µg g⁻¹ egg) | TCA (%RSD, N=3) | Alkali Dry Ashing (%RSD, N=3) |
|-------------------------|-----------------------------------|-----------------|-------------------------------|
| Egg 1                   | 1.05 (4.8)                        |                 | 0.63 (5.3)                    |
| Egg 1 + (10 µg I)       | 11.3 (1.2)                        |                 | 6.10 (10)                     |
|                         | (%Recovery = 102%)                |                 | (%Recovery = 48%)             |
| Egg 2*                  | 2.23 (2.6)                        |                 | 1.39 (15)                     |
| Egg 3*                  | 1.95 (4.7)                        |                 | 1.06 (6.5)                    |
| Egg 4*                  | 13.3 (3.3)                        |                 | 6.47 (3.6)                    |
| (Iodine-enriched egg)** |                                   |                 |                               |

*Significant difference, P<0.05 one-tailed t-test within the sample

**Labeled with (Iodine content of 13 µg/g egg)
Figure Captions

Fig. 1 Flow-based Sandell-Kolthoff colorimetric system. PP: peristaltic pump; IV: six port injection valve with sample loop 0.25 mL; RC: reaction coil (200 cm, 1.32 mm i.d.); D: detector; W: waste

Fig. 2 Egg samples after treatment by (a) adjusting pH; (b) salting out; (c) SDS; and (d) TCA

Fig. 3 Signals of iodine obtained from (a) sample treated with SDS and (b) sample treated with TCA using continuous flow injection system. (1) Deionized water; (2) iodine solution at 500 µg I L⁻¹; (3) treated deionized water; (4) treated iodine solution at 500 µg I L⁻¹; (5) treated egg samples; and (6) treated iodine spiked egg sample at 500 µg I L⁻¹.

Fig. 4 Recovery of iodine (500 µg I L⁻¹) from egg samples treated with different volumes of TCA.

Fig. 5 Recovery of iodine (500 µg I L⁻¹) from egg samples treated with TCA (400 µL of 1 g mL⁻¹) at different incubation time.
**Fig. 1** Flow-based Sandell-Kolthoff colorimetric system. PP: peristaltic pump; IV: six port injection valve with sample loop 0.25 mL; RC: reaction coil (200 cm, 1.32 mm i.d.); D: detector; W: waste.
Fig. 2 Egg samples after treatment by (a) adjusting pH; (b) salting out; (c) SDS; and (d) TCA
Fig. 3 Signals of iodine obtained from (a) sample treated with SDS and (b) sample treated with TCA using continuous flow injection system. (1) Deionized water; (2) iodine solution at 500 µgI L\(^{-1}\); (3) treated deionized water; (4) treated iodine solution at 500 µgI L\(^{-1}\); (5) treated egg samples; and (6) treated iodine spiked egg sample at 500 µgI L\(^{-1}\).
Fig. 4 Recovery of iodine (500 µg L⁻¹) from egg samples treated with different volumes of TCA.
Fig. 5 Recovery of iodine (500 µg L⁻¹) from egg samples treated with TCA (400 µL of 1 g mL⁻¹) at different incubation time.
Graphical Index