

The stability of double fortification of salt with iodine and iron in different storage conditions

¹Helmyati, S., ²Narendra, Y. H., ¹Septi, P., ¹Rochyana, I. and ¹Endri, Y.

¹School of Health Nutrition, Faculty of Medicine, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia

²Health Analyst, Polytechnic of Health, Ministry of Health, Ngadinegaran MJ III/62, Yogyakarta, Indonesia

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Abstract

Iodine deficiency disorders (IDD) and iron deficiency anemia (IDA) often coexist. Double fortification with iodine and iron can be applied for coping those two major micromineral deficiencies. Unfortunately, iodine is easily oxidized. This study was conducted to identify the stability of double fortification of salt with iron (NaFeEDTA) and iodine (potassium iodate/KIO₃) in different storage conditions. The concentration variations were 0% (control), 0.02%, 0.05%, and 0.1% of NaFeEDTA in fortified salt. All samples were stored for 28 days in opened and closed storage. Iodine concentration was analyzed with iodometric titration method at day of 0, 7, 14, 21, and 28. The results showed iodine retention after 28 days of storage declined. In opened storage, iodine retention in NaFeEDTA 0%, 0.02%, 0.05%, and 0.1% was 61.76%, 60.59%, 69.48% and 76.76%, respectively from baseline iodine content. Meanwhile, in closed storage, the iodine retention on the same concentrations was higher, namely 83.40%, 82.96%, 84.33%, and 82.62%, respectively. Both of storage conditions showed there was no effect of concentration variations of NaFeEDTA on iodine stability ($p > 0.05$). Double fortification with iodine and iron in form of NaFeEDTA had good stability, especially if salt was stored in closed condition.

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Introduction

Food fortification has been relied as an effective way to overcome micromineral deficiencies such as salt iodization as an effective and well-established intervention strategy for combating iodine deficiency disorders (IDD) (ICCIDD *et al.*, 2001) and iron fortification for combating iron deficiency anemia (IDA) (Le *et al.*, 2006). Iron fortification was less costly than supplementation (Baltussen *et al.*, 2004). Thus, the double fortification of salt with iodine and iron can be applied for coping the two major micromineral deficiencies in Indonesia; IDD and IDA, that often coexist. The national Total Goitre Rate (TGR) in Indonesia was 11.0% (Ministry of Health of Indonesia, 2003) while the prevalence for anemia was 11.9% (Ministry of Health of Indonesia, 2008).

Salt was cheap, easily obtained, and consumed every day by all concentrations of societies in all economic concentrations. It could be a potential food vehicle for iron fortification. However, iron fortification can cause several biophysicochemical modifications. In addition, the interaction between iodine and iron resulted in decreasing iodine bioavailability due to iodine loss (Diosady and Mannar, 2000). That was caused by redox potential of

iron, moisture, impurities, environmental humidity, and temperature (Shelswell, 2003).

Iron fortificant was hygroscopic and was directly affected by the concentration of water vapor in the environment. The change of atmospheric temperature of material will affect the iron reactivity with that material (Akhtar and Anjum, 2007). During double fortified salt storage and distribution, almost of 25% Fe³⁺ from NaFeEDTA was reduced to Fe²⁺ (Oshinowo *et al.*, 2007).

One similar research had ever been done by Rao (1994). In that study, the source of iron was ferrous sulphate with sodium hexamonophosphat (SHMP) and polyphosphate as a stabilizer. The results showed the most stable compound was the salt with addition of iodine SHMP 1% (over 6 months), and the increase concentration of SHMP did not cause the decrease of the iodine stability. From that study, it was also known polyphosphate salt stabilizer caused discoloration. All of biophysicochemical modifications occurring in iron fortification depend on many factors, including the source of iron.

NaEDTA has been known as a stabilizer forming a stable complex with Fe. A previous study showed absorption of Fe from NaFeEDTA was significantly better than other iron fortificants, and it can minimize the color changes of fortified food (Ballot, 1989). The

*Corresponding author.

Email: siti_helmyati@yahoo.com

Tel/fax: +6227454775

purpose of this study was to assess the iodine stability of salt fortified by potassium iodate (KIO_3) and NaFeEDTA in opened and closed storage condition.

Materials and Methods

This study was pre-post with control design, conducted at the Nutrition Laboratory, Faculty of Medicine, Universitas Gadjah Mada, started from May till June 2009. In this study, there were 8 experimental units (one control group and three treatment groups, each in opened and closed storage conditions). The experiments were performed in duplicate.

Salt preparation

Salt was prepared as follows: salt was washed with distilled water twice, drained, then dried with sunlight and baked. Before fortified, the level of Fe in refined salt was measured by using AAS (Atomic Absorption Spectrophotometry) method. The moisture (water content) in salt was determined by thermogravimetric method. NaFeEDTA used in this study was made with the method reported by Layrisse and Martinez-Torres (1977).

The amount of salt fortified with KIO_3 was 5 kg. We used the mixture methods consisting of two steps, making premix and mixing. A total of 300 mg KIO_3 was dissolved in 25 mL distilled water. KIO_3 solution was put in the sprayer and sprayed within 1 kg of salt (spray mixing). Salt was manually stirred with mixer for 10-15 minutes. Then, 1 kg salt (premix) was mixed into 4 kg unfortified salt, stirred manually and homogenized with mixer for 10-15 minutes.

Salt that had been fortified with KIO_3 was divided into four parts (each 1,250 g), then fortified with different concentrations of NaFeEDTA. There were four variations of NaFeEDTA concentration, namely 0%, 0.02% or 200 ppm (200 mg/1 kg), 0.05% or 500 ppm (500 mg/1 kg), and 0.1% or 1000 ppm (1000 mg/1 kg). Fortified salt with NaFeEDTA and KIO_3 was weighed each 125 g and put into the plastic glass. Then, plastic glass was placed in trays and stored in the laboratory in opened and closed conditions.

Iodine analysis

All experimental units were stored for 28 days. During the storage, periodic iodometric titration was performed at day of 0, 7, 14, 21, and 28, respectively to determine the iodine content. The principle of iodometric method was the occurring of color change after titration (Saksono, 2003). In iodometry, salt was reduced by potassium iodide 10%, resulted in iodine which was further titrated with standard solution of sodium thiosulfate. In this iodometric titration,

we used starch 1% as an indicator. During the first titration, solution had dark blue color, but at the end of titration became colorless. That change was led by the concentration change of I_2 into 0 (noI) (ICCIDD et al., 2001). According to Indonesian standard (SNI) 1992, the procedure of iodometric titration was as follows; about 25 g of salt was accurately weighed, then dissolved in 125 mL distilled water. The salt was dissolved in 2 mL phosphoric acid, 2 mL 10% KI and 2 mL starch 1%. The color after the addition of those three solutions into salt was dark blue. The final step of the iodimetric reaction was titration by using 0.005 N $\text{Na}_2\text{S}_2\text{O}_3$ until the blue color disappeared.

Statistical analysis

We used one-way ANOVA for data which was normally distributed and had equal variance to determine the effect of NaFeEDTA concentration variation on the stability of KIO_3 . A repeated ANOVA was performed to determine the effect of storage duration on the stability of KIO_3 .

Results and Discussion

Characteristics of salt and NaFeEDTA

Iron is one of reductor agents that can cause iodine loss in salt. Total iron in NaFeEDTA was analyzed to estimate the amount of iron if we consume a certain amount of this fortified salt with NaFeEDTA (Table 1). Physical characteristics of salt used in this study were white, powder and dry. Average water content in salt (WB) was 3.41%, and the average moisture content (db) was 3.53%. The characteristics of NaFeEDTA were pale yellow in color, powder, and with a molecular weight of 421.10 mol/g.

The effect of NaFeEDTA concentration and the duration of storage on the iodine stability

The effect of each NaFeEDTA concentration on the iodine stability changed with the increasing duration of storage. The change in iodine content was basically influenced by two factors, namely storage duration and the concentration of NaFeEDTA. In opened storage, NaFeEDTA 0.1% showed the best effect on the stability of iodine content followed by NaFeEDTA 0.05% and 0.02% (Table 2). As longer the storage duration, the iodine tended to decrease. Statistical tests showed that the effect of NaFeEDTA concentration and the duration of storage on the stability of KIO_3 content did not differ significantly ($p > 0.05$). In closed storage, there was also no significant difference in iodine retention of salt fortified by some variations of NaFeEDTA concentration (Table 3).

Decline in iodine concentration can be associated

Table 1. Analysis of iron concentrations in salt and NaFeEDTA

Sample description	Fe (mg/L)
1.1482 g salt dissolved in 100 ml aquadest	0.002
0.5027 g NaFeEDTA dissolved in 100 ml aquadest	0.0571

Table 2. Mean percentage of iodine retention in opened storage

Day	Concentration variation of NaFeEDTA			
	0%	0.02%	0.05%	0.10%
Day 0	100	100	100	100
Day 7	61.28	82.89	83	93.7
Day 14	76.42	70.28	75.28	93.38
Day 21	66.4	71.17	76.99	87.52
Day 28	61.76	60.59	69.48	76.76

Table 3. Mean percentage of iodine retention in closed storage

Day	Variation concentration of NaFeEDTA			
	0%	0.02%	0.05%	0.10%
Day 0	100	100	100	100
Day 7	79.62	77.07	86.54	78.44
Day 14	89.49	77.27	85.94	84.62
Day 21	89.26	84.67	86.68	83.00
Day 28	83.40	82.96	84.33	82.62

with impurities in the salt. In this study, the number and type of metal impurities in the salt was not examined. According to Marihati and Prasetya (2002), the iodine stability in salt was influenced by the purity of salt or the existing of impurities in salt, water content, and heating. Impure compounds in salt include hygroscopic compounds e.g. $MgCl_2$, $CaCl_2$, $MgSO_4$ and $CaSO_4$, and some reducing agents such as Fe, Cu, Zn and organic compounds (Bahruddin *et al.*, 2003). Reductor in the salt will cause the release of iodine (I_2) because KIO_3 is strong oxidizing agent. This reaction is accelerated by an acid environment that comes from impurities in salt (Marihati and Prasetya, 2002). Iodine is easy to sublime and lose to the air through diffusion (Diosady *et al.*, 1998).

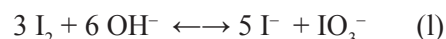
There was no effect of NaFeEDTA concentration variations on the iodine stability. It showed NaFeEDTA is stable in the binding of iron, as a consequence, iron ion cannot react with KIO_3 . Fe^{3+} ion in NaFeEDTA forms a strong complex with EDTA and has a high stability. Metal ion can be bound by NaEDTA and then form complex compounds with a certain stability, depending on the log value of equilibrium constanta (Nayak and Nair, 2002).

As mentioned above, 0.1% NaFeEDTA was able to maintain iodine content, better than other concentrations, although the result of this study was not statistically significant. EDTA is a hexadentat chelator which is able to combine stoichiometrically with every metal in the periodic table. EDTA is effective to chelate metal ion, because their stability with the metal ion was constant. This strong chelation is affected by pH, molar ratio of EDTA and metal ion, and the existence of other metal ion which are able to form complexes with EDTA. The complex stability of EDTA-metal is varied, depends on the kind of metal. Important metal such as Fe^{3+} has the highest stability ($\log K = 25.1$) (Bothwell and MacPhail, 2004). Na^+

has the lowest stability (1.7) compared with other metals such as Cu^{2+} (18.4), Zn^{2+} (16.1), Fe^{2+} (14.6), Ca^{2+} (10.6), and Mg^{2+} (8.7) (Bassett *et al.*, 1994).

The low stability between Na ion and EDTA may be replaced by other metal ion having higher stability, such as Cu, Fe, Ca, and Mg. Fe ion in salt was a reducing agent which disturb the stability of iodine. If EDTA is bound to iron, the iron ion cannot react with iodine, so that the iodine loss can be minimized. Therefore, the high concentration of NaFeEDTA can retain more iodine content than that in the lower concentration. The lack of storage duration effect on the iodine stability was caused by two factors: 1) there were some data showing higher concentrations of iodine compared to the previous measurement. Normally the longer of storage time, the more iodine will lose. 2) limited measurement time (only seven days away for 28 days) led to less significant in differences of iodine content between the measurement times.

In previous study conducted by Saksono (2002), there were also fluctuations of KIO_3 content in salt, which KIO_3 concentrations on a longer storage duration were higher than those stored in shorter duration. The process of iodate equilibrium was caused by the environmental changes. This equilibrium led to the occurrence of other reactions that form iodate and may more dominant than the reaction of iodate reduction. One reaction that forms iodate ion is below:



Based on the Nernst equation, $G^\circ = -162.12 \text{ kJ / mol}$ with $n = 6$. This reaction can take place spontaneously when there was enough I_2 and supported by alkalyne environment.

After one month storage, the final iodine content was 30.82 – 39,28 ppm. This iodine content was still eligible according to the regulation of Minister of Health of Indonesia, no. 165/Menkes/SK/II/1986 on 26 February 1986 about iodized salt. It stated the iodine content in domestic iodized salt in production and distribution should be 30-80 ppm (Soeid *et al.*, 2006). Apart from the influence of storage condition and NaFeEDTA concentration variations, the iodine stability of salt in storage was influenced by several factors such as pH, temperature, humidity, impuritis, and ways of packaging (Diosady, 1998). The potential factor in developing a stable complex between iodine and iron in salt was the iodine stability in the presence of iron after storage. It was affected by the purity of salt, water content, and environmental factors, including humidity and temperature (Rao, 1994).

Organoleptic properties, food safety, and bioavailability of iron in the double fortification of salt with KIO₃ and NaFeEDTA

The addition of NaFeEDTA in salt with the lowest concentration (0.02%) had already led to change the color of salt even prior to the storage process (unpublished data). NaFeEDTA caused fewer organoleptic changes than other iron fortificants (Bothwell and MacPhail, 2004). NaFeEDTA met the specifications of JEFC as food fortificant, that had the bright yellow color. In this study, NaFeEDTA we made was tasteless, odorless, and pale yellow color.

NaFeEDTA has high biological value of iron but less than ferric gluconate. Relative biological value (RBV) of ferric gluconate was 98% while RBV of NaFeEDTA was 86% (Lysionek *et al.*, 2001). Daily iron requirement for Indonesian adult males was 13 mg and that for adult women was 14 mg (Ministry of Health, 2004). The permitted intake of EDTA was 2.5 mg EDTA/kg body weight/day (Bothwell and MacPhail, 2004) while the recommended iron intake from NaFeEDTA was 0.2 mg /kg body weight/day (Hurrell and Egli, 2005). The estimation intake of salt per day was 10 – 20 g (Rao, 1994), the daily intake of NaFeEDTA was 10 – 20 mg for salt with NaFeEDTA 0.1% (1000 mg NaFeEDTA in 1 kg salt). This amount is safe compared to recommendation of iron and EDTA consumption.

Conclusions

Double fortification of salt with iodine and iron in the form of NaFeEDTA had good stability especially if stored in closed condition. It needs further study to know the effectiveness of this double fortified salt for overcoming micronutrient deficiencies.

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