

## Stability of Micronutrients (Vitamin A, Iron And Iodine) Content in Fortified Rice

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**Abstract:** More than 2 billion people in the world today suffer from micronutrient deficiencies caused largely by a dietary deficiency of vitamins and minerals. The public health importance of these deficiencies lies upon their magnitude and their health consequences, especially in pregnant women and young children, as they affect fetal and child growth, cognitive development and resistance to infection. Although people in all population groups in all regions of the world may be affected, the most widespread and severe problems are usually found amongst resource poor, food insecure and vulnerable households in developing country. Some efforts that have been done to solve the problem such as micronutrient fortification in rice, because it is the staple food consumed by more than 90% Indonesia's population. The objectives of this research were determined and evaluated the stability of micronutrient level during rice fortification and cooking. The results showed that micronutrients decreased during rice fortification and cooking. The raw-fortified rice contained 38.52 - 59.91mg/L of iodine, 0.92-1.79mg/L of vitamin A and 15.64 - 48.39 mg/L of iron depending on the concentration of the coating material used. After cooking, the fortified rice contained 3.00- 3.93mg/L of iodine, 0.43 - 1.0 mg/L of vitamin A and 12.92-19.66 mg/L of iron. The levels of micronutrients, although experienced significant losses, still meet the daily needs according to the Regulation of World Health Organization.

**Keywords:** Rice, micronutrient, food fortification.

## INTRODUCTION

Essential micronutrient deficiency widely affects more than a third of the world's population, especially in developing countries including Indonesia. There are three major micronutrients deficiency in Indonesia, namely iodine deficiency disorders (IDD), iron deficiency anemia (IDA) and vitamin A deficiency (VAD). Around 40% of Indonesia's population suffered from anemia, with a total of 100 million people including people with IDD and VAD. In Indonesia, the prevalence of anemia increased by 61 percent and 65 percent at infants aged 6 and 12, respectively, and increase by 31 per cent at malnourished children. Lack of essential micronutrients results in inability to learn well, mental retardation (physical and mental development disorders), poor health, low work capacity, blindness, goitre and premature mortality and potential suffered from anemia. These lead to loss of social and economic potential of society, accounted for around 5% of gross domestic product (GDP) of a country. Micronutrient deficiencies are often found in countries with rice as a staple food, including Indonesia Clugston *et al.*, [1] Allen *et al.*, [2] Bernatal *et al.*, [3] Endi [4] Wirth *et al.*, [5].

Micronutrient deficiency problem in Indonesia can be overcome through some approaches such as diversification of food consumption, supplementation and food fortification. Food fortification is more effective than the other two as diversification of food consumption and supplementation are relatively expensive to be applied to the lower economic community Susilowati [6] Nair *et al.*, [7].

Rice fortification is a promising approach to combat micronutrient deficiency problem in Indonesia as rice is the staple food consumed over 90% of Indonesia's population with a consumption of around 150 kg / capita, or about 200 g / day. Generally, rice meets 22% of the total energy required and contributes to the largest percentage in the fulfillment of calories and protein. However, low energy and protein intake are common problem faced by rice-consuming countries. Food and Agriculture Organization (FAO) noted that at least half of the population in South Asia do not have enough energy intake to meet daily activities Allen *et al.*, [2] WHO [8] Thankachan [9].

Food fortification is generally used to treat and control the problem of micronutrients in the medium

and long term. The main objectives of food fortification are increasing consumption levels of nutrients and improving nutritional status of the population or community. The fundamental role of food fortification program are (1) to assure that required micronutrients are available and consumed in sufficient quantities; (2) to increase the level of consumption of nutrients and to improve the nutritional status of the population Allen *et al.*, [2] Nair *et al.*, [7].

The problems encountered in rice fortification is to retain the content of micronutrients in rice during processing and storage. In order to maintain the content of micronutrients, microencapsulation is applied to provide protection against adverse environmental conditions such as high temperature, humidity, light and reactions with other materials that are not desirable. In addition, by microencapsulation, the release rate of the active ingredient can be controlled (controlled release) so as to maintain current levels of micronutrients Allen *et al.*, [2] Yuliani *et al.*, [10] Yuliani [11] Thankachan [9]. The objective of this research is to evaluate the stability of encapsulated micronutrients during rice fortification as well as during handling, washing and cooking.

## MATERIALS AND METHODS

### Raw Materials and Chemicals

The main material used in this study consisted of rice (IR-64) obtained from the State Logistics Agency (Bulog), Indonesia, tofu whey from tofu industry waste in Bandung, maltodextrin DE 16.5-19.5 (Merck, Darmstadt, Germany), potassium iodide (Merck, Darmstadt, Germany), potassium iodate (Merck, Darmstadt, Germany), Iron-fumarate (Merck, Darmstadt, Germany) and vitamin A/retinol acetate (Merck, Darmstadt, Germany), 1% starch, concentrated phosphoric acid (Merck, Darmstadt, Germany), acetonitrile-dichloromethane = 5:3 v/v (Merck, Darmstadt, Germany) and methanol for HPLC (Merck, Darmstadt, Germany).

### Preparation of coating material for rice fortification

Tofu whey powder was prepared by drying of tofu whey precipitate using a spray dryer (Lab Plant 05, Germany). The coating material is a mixture of tofu whey powder (6%), maltodextrin (14%),  $KIO_3$  (7.5%), Fe-fumarate (1000 mg/L), vitamin A (1000 mg/L) and distilled water (71%). The mixture was homogenized using an ultraturrax homogenizer (Micra D9, 40233, Germany) with a speed of 11000 rpm for 15 seconds, then hydrated in the cooling room at 4°C overnight. The coating solution was then dried by means of spray dryer at an inlet temperature of 170°C with the feed flow rate of 15 mL / min. The coating powder was collected and kept in plastic bags for further study.

### Determination of micronutrient content

Determination of micronutrient was carried out on samples of un-cooked and cooked rice (boiled).

Iodine was determined by uv-vis spectrometry ((UV-2600, Shimadzu), iron was determined by Atomic Absorption Spectrophotometry (AA-7000, Shimadzu), and vitamin A was determined High Performance Liquid Chromatography (LC-20 AT Shimadzu).

### Rice fortification with iodine, iron and vitamin A.

Three different concentration of coating materials (7%, 14% and 21%) were prepared by dispersing 70 g, 140 g and 210 g of the coating material in 1000 mL of distilled water, respectively. Fortification is done by spraying the coating suspension onto rice during milling process (rice polishing). Considering the rice consumption per person of 300-400 g / day, the spraying was set at a flow rate of 0.5 mL / kg / min. Fortified rice was collected in a container, weighed and stored in plastic bags for further testing.

### Determination of iodine as $KIO_3$ using uv-vis Spectrometry

Sample of fortified rice (2.5 g) was mixed with 50 mL distilled water in a 100 mL volumetric flask. A solution of lead acetate was added dropwise into the flask until giving no turbidity and distilled water was added to make a 100 mL solution. After shaking, the solution was filtered and the filtrate was separated into a 100 mL volumetric flask. Sodium phosphate solution 8% was added 10 mL, and the volume was topped up to 100 mL mark by distilled water. After filtering, the filtrate was poured into five of 25 mL volumetric flasks for preparation of 5 different concentrations of  $KIO_3$  standard solution. Two milliliter of filtrate was placed into each flask and added with 50 ppm of  $KIO_3$  standard solution (0.5 mL, 1 mL, 1.5 mL, 2 mL, and 2.5 mL in each flask). Ten milliliter of solvent and 0.5 mL of 0.5% diphenylamine were added into each flask. The mixture was shaken and another 10 mL of solvent was added. The volume of mixture was then adjusted into 25 mL mark with distilled water. The absorbance (A) of all solutions was measured at a maximum wavelength (460 nm) to construct the calibration curve (absorbance vs. concentration (mg/L)). The absorbance of all samples were then measured and concentration of the  $KIO_3$  in the samples were determined by absorbance vs. concentration correlation curve.

### Determination of iron content

Sample of fortified rice (0.5 - 1.0 g) were placed into a beaker, added with 10 mL  $HNO_3$  and destructed. Destruction was done until a clear and concentrated solution was produced. Once destruction was completed, the solution was transferred into a flask. Standard solutions were prepared by diluting the standard solution of iron 100 mg / L into series of concentration (0.25, 0.50, 0.75, 1.0, 1.25 and 1.5 mg / L). The absorbance of the standard solution and the samples were measured at a wavelength of 248.3 nm. A standard calibration curve was constructed by plotting the absorbance versus the concentration of iron in the

standard solution. Iron concentration in the samples was determined using a regression equation developed from the standar calibration curve.

#### Determination of vitamin A content

Sample of fortified rice was weighed accurately equivalent to vitamin A 2500 UI and placed into a 100 mL beaker glass. Twenty milliliter of methanol was added into the beaker and the mixture was stirred for 2 to 2.5 h at room temperature. Samples must be covered during the process to avoid direct exposure of light. The methanol extract was then filtered using a membrane filter of 0.22 and 0.45 micro meter (Cameo 3N, Nylon from Scharlau) and it transferred into a 25 mL volumetric flask for HPLC analysis. The optimum conditions of HPLC were as follows: a column of 150 x 3.9 mm I.D. ; mobile phase acetonitrile-tetrahydrofuran-water (55-37-8, v / v); flow rate of 1.5 mL / min; infrared photodiode detector arrays with NEC power mate 386 / 33i; injection volume of 2 mL; wavelength ( $\lambda$ ) 325 nm; acquisition of 1 second and a resolution of 4.8 nm.

## RESULTS AND DISCUSSION

Encapsulation of micronutrient was done by spray drying with the properties of encapsulate powder as seen in Table-1. Considerable losses of micronutrient were observed during spray drying, ranging from 60.0% to 93.63% from initial concentration prior to spray drying (iron 1000 mg/L, vitamin A 1000 mg/L and  $KIO_3$  7.5%).

#### Iodine content in fortified rice

Iodine content was analysed both in raw and boiled fortified rice. Fortified rice showed low concentrations of micronutrient with losses from 89.80% to 94.99% (Table-2), which might be associated with processing conditions of, washing and cooking.

Extensive losses of iodine might occurred during series of steps of cooking. Washing of rice before cooking might result in dissolution of micronutrient and washed away. Fidler [14], found that Micronutrients in fortified or enriched rice might be lost during storage, washing, and cooking with total loses of around 60%. Application of coating can increase the survival of the micronutrients against washing and cooking up to 10 – 30 %. However, boiling rice with excessive water might cause a higher losses of micronutrients more than 80%. In the form of  $KIO_3$ , iodine deposited onto the rice surface was easily subjected to internal dan external factors of deterioration, such as humidity, temperature, storage time, type of packaging, the presence of metal mainly iron, moisture content, light, acidity, and the presence of reductor substances and hygroscopic materials. At high temperature,  $KIO_3$  would be degraded into  $I_2$  and  $I_2$  would be evaporated during storage and cooking [18-20, 10].

According to Diosady *et al.*, [12],  $I_2$  formed from the decomposition of  $KIO_3$  will quickly evaporated at room temperature and even disappeared entirely at 40°C. Microencapsulation techniques are able to maintain the stability of  $KIO_3$  approximately 10-30%. The presence of iron in the form of Fe (II) fumarate, in the coating formulation will accelerate the reduction of iodine during rice processing because  $Fe^{2+}$  in Fe (II) fumarate is a reducing agent that breaks down  $KIO_3$  into  $I_2$ . The iodine content of the fortified rice still meet the needs of daily intake of iodine according to WHO, ie. 40-120  $\mu g$  / day for children, 150  $\mu g$  / day for adult, and 25  $\mu g$  / day and 150  $\mu g$  / day for pregnant and lactating mothers, respectively Diosady *et al.*, [12] Wegmuller *et al.*, [13].

#### Iron content in fortified rice

The results showed decreases in iron levels after spray drying, rice milling and cooking with the higher losses at the higher concentration of coating. Decreases in iron content in raw and cooked fortified rice were shown in Table 3. Losses of iron in cooked rice might be mainly attributed to washing process before cooking. Fortification of rice by using a coating method was very fragile and the micronutrient can be washed away during washing and cooking Fidler [14]. Microencapsulation gave an advantage, such that the capsule wall acted as a barrier, preventing the interaction between iron and iodine or between iron with air Diosady *et al.*, [12] Soeid *et al.*, [15]. Without encapsulation, fortified rice might lost up to 100% fortificants, depending on cooking time and water quantity used for washing and cooking. Another cause of iron decrease in the rice processing was the abundant phytic acid in the outer layer of rice (aleurone) Indrasari [16] Endi [4]. The iron content of the fortified rice still meet the needs of daily intake of iron according to WHO. And Regulation of Health Ministry of Republic of Indonesia, ie 7-10 mg / day for children, 8-18 mg / day for adult, and 27 mg / day for pregnant woman WHO [8].

#### Vitamin A content in fortified rice

Vitamin A content in fortified rice decreased with the advance of rice processing (Table-4). High temperature during spray drying might reduce Vitamin A content in the encapsulated coating material. Furthermore, washing of rice before cooking leached out the fortificant deposited onto rice surface. Long high temperature exposure during rice boiling might also result in the destruction of vitamin A, Raileanu *et al.*, [17] Wegmuller *et al.*, [13].

Vitamin A is easily oxidized, rather stable when heated in a vacuum in the absence of light, unstable in the presence of ultraviolet, and fairly stable in alkaline conditions. The presence of metals can accelerate the oxidation of vitamin A. The present work showed that among three micronutrients fortified into rice, iodine suffered the highest losses. Interaction

between vitamin A and iron seemed synergistic. Addition of vitamin A can improve utilization and

status of iron and vitamin A in the body as compared to addition of vitamin A alone or iron alone Endi [4].

**Table-1: Characteristics of encapsulated micronutrient**

Characteristics	Value
Moisture content	3,22 %
Water Activity	0,254 %
Water solubility	86,96 %
Iron content	330 mg/L
Iodine content	4,54 %
Vitamin A content	63.7 mg/L

**Table-2: Iodine content of fortified rice and boiled rice**

Concentration of coating (% w/v)	Iodine content as KIO <sub>3</sub> (mg/L)		Losses (%)	Iodine content as KIO <sub>3</sub> (mg/L)		Losses (%)
	Coating material	Rice		Rice	Boiled rice	
7	315	38.52	87.77	38.52	3.93	89.80
14	630	47.84	92.41	47.84	3.42	92.85
21	945	59.91	93.66	59.91	3.00	94.99

**Table-3: Iron content of fortified rice and boiled rice**

Concentration of coating (% w/v)	Iron content (mg/L)		Losses (%)	Iron content (mg/L)		Losses (%)
	Coating material	Rice		Rice	Boiled rice	
7	23.1	15.64	32.29	15.64	12.92	17.39
14	46.2	24.61	46.73	24.61	16.22	34.09
21	69.3	48.39	30.17	48.39	19.66	59.37

**Table-4: Vitamin A content of fortified rice and boiled rice**

Concentration of coating (% w/v)	Vitamin A content (mg/L)		Losses (%)	Vitamin A content (mg/L)		Losses (%)
	Coating material	Rice		Rice	Boiled rice	
7	4.46	0.92	79.37	0.92	0.43	53.26
14	8.92	1.11	87.56	1.11	0.56	49.50
21	13.38	1.79	86.62	1.79	1.00	44.15

## CONCLUSION

Micronutrients fortified into rice suffered from losses during several stages of rice processing. Spray drying contributed the lowest/highest losses of micronutrient, while washing and cooking gave 60.0% to 93.63% micronutrient losses. Although considerable losses of micronutrients were found during rice fortification and cooking, to the micronutrient content still meet the needs of daily micronutrient intake according to standards of the Indonesia Ministry of Health and WHO.

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