Formulation and Effect of Iron Fortified Instant *Bose* Corn on Addressing Anemia among Adolescent Girls in Kupang, Indonesia

Ludia Simuruk Gasong¹, Evy Damayanthi², Sri Anna Marliyati², and Drajat Martianto²

¹Department of Food Technology, Kupang State Agricultural Polytechnic, Kupang 85148, Indonesia ²Department of Community Nutrition, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia

ABSTRACT: This study aimed to develop Fe-fortified instant *Bose* corn (IBC), a traditional Timorese food, as a strategy to address anemia among adolescent girls in Kupang, Indonesia. Instant corn and cowpea grits were made to shorten the preparation time with various times of soaking (IBC1=10 min; IBC2=15 min; IBC3=20 min) to select the best formula. The selected IBC was then fortified with NaFeEDTA·3H₂O and nutrient content was evaluated. In the intervention study, a pre-post controlled trial was applied to 40 anemic adolescent girls aged $16 \sim 19$ years. Subjects with severe anemia (Hb= $8.5 \sim 10.4$ g/dL) received the fortified selected IBC, whereas those with moderate anemia (Hb= $10.5 \sim 11.5$ g/dL) received the unfortified selected IBC. A total of 100 g IBC were provided three times/week for 2 months. The hemoglobin and soluble transferrin receptor (sTfR) levels were measured at baseline and endline, respectively. IBC3 produced the highest acceptance level as compared to other formulas. The fortified IBC3 met 127.4% recommended dietary allowances for iron. Moreover, both fortified and unfortified IBC3 significantly increased the hemoglobin levels of adolescent girls with the higher improvement found in the group of fortified IBC3 (1.73 ± 1.21 g/dL; *P*<0.05). However, the beneficial effects of the fortified IBC3 for only 2 months did not adequate to improve sTfR levels. Nevertheless, this study suggests that Fe-fortified IBC may be effective in preventing anemia among adolescent girls in Kupang, Indonesia.

Keywords: adolescent, anemia, fortification, hemoglobin, iron

INTRODUCTION

Nutritional anemia is a major problem in Indonesia, particularly in Kupang, East Nusa Tenggara (ENT). The overall anemia prevalence in Indonesia was 23.7%, with adolescent girls having the highest prevalence at 48.9% in 2018 (Ministry of Health of Republic of Indonesia, 2018). A recent study revealed 64.5% of adolescent girls to be anemic in Kupang, ENT (Djogo and Letor, 2022). Anemia in adolescent girls has been linked to a delay in cognitive development, physical and mental growth, and even sexual maturity (Sen and Kanani, 2006). For longterm consequences, anemia increases the risk of low birth weight, miscarriage, bleeding, and maternal death (Imdad and Bhutta, 2012; Bihoun et al., 2017).

Iron deficiency anemia (IDA) is the most common anemia in all stages of life (Masfiah et al., 2021). Low intake of micronutrients, including iron, folate, and vitamin B12, play a significant role in IDA. People with anemia are encouraged to routinely consume iron supplements and iron-rich foods (Imdad and Bhutta, 2012). The Indonesian government has freely provided iron supplementation for adolescent girls and pregnant women as a strategy to prevent anemia; however, the current prevalence is still high and needs more effort (Barkley et al., 2015). Some barriers have been reported including inadequate plan and supply management, lack of awareness of anemia, and supplement side effects, which lead to low adherence of the supplement consumption (Barkley et al., 2015; Alfiah et al., 2020). Thus, dietary approaches have been proposed as a sustainable strategy for addressing anemia (Aspuru et al., 2011; Sun et al., 2018).

Bose corn is a Timorese traditional food that contains corn, beans, and coconut milk (Gasong et al., 2018). This food originates from ENT and is commonly consumed as a rice substitute (Eka Yulianti et al., 2022). Our preliminary study showed that *Bose* corn is the most preferred and often consumed local food among adolescent girls in

Correspondence to Ludia Simuruk Gasong, E-mail: gasongludi@yahoo.com

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Author information: Ludia Simuruk Gasong (Professor), Evy Damayanthi (Professor), Sri Anna Marliyati (Professor), Drajat Martianto (Professor)

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Kupang (Gasong et al., 2018). However, the preparation time is relatively long (4 h) and it is required to be shortened. Therefore *Bose* corn that instantly prepared was se-3H₂O (416 57 mg

time is relatively long (4 h) and it is required to be shortened. Therefore, *Bose* corn that instantly prepared was selected as local food to address IDA among adolescent girls in Kupang. Since the iron content of *Bose* corn was relatively low ($1 \sim 2 \text{ mg}/100 \text{ g}$) (Eka Yulianti et al., 2022), the present study aimed to develop iron fortified instant *Bose* corn (IBC) with a short preparation time to improve anemia status among adolescent girls.

MATERIALS AND METHODS

Design

The two major phases of the current investigation were IBC formulation and intervention. A completely randomized design and a pre-post controlled design were used for IBC formulation and the intervention, respectively. The first phase, i.e., IBC formulation and analysis was done at Southeast Asian Food and Agricultural Science and Technology Laboratory, and Laboratory of Food Analysis, IPB University. The second phase, i.e., IBC intervention was conducted at Hidayatullah Islamic Senior High School in the Sub-district of Batakte, Kupang.

IBC formulation

IBC preparation: The IBC's formulations were prepared using local corn (60%), cowpea (10%), coconut milk (20 %), cornstarch (2%), salt (3%), and sugar (5%) according to Husain (2006) with little modifications and difference in soaking time of formulations were compared. Instant corn and cowpea grits were used to shorten the preparation time. The instant corn and cowpea grits were prepared by grinding the dried granules that had previously been soaked into 1% Na-citrate solution for 10 min, steamed at 100°C for 10 min, and frozen at -40°C for 44 h (Eka Yulianti et al., 2022). The instant corn and cowpea grits were then soaked into water (ratio of 1:10) with various soaking time (IBC1=10 min, IBC2=15 min, and IBC3=20 min). The soaking times were determined according to a previous study which used 15 min to soak the grits (Husain, 2006). This time was then used in a preliminary study with a trial-error approach that found more than 20 min of soaking time might produce the same result as only 20 min. The soaked grits were subsequently boiled for 10 min at 90°C, while coconut milk, cornstarch, salt, and sugar were added, hence the IBC was obtained.

Hedonic test: A total of 49 semi-trained panelists conducted a hedonic test. The test used a five-scale score (1=dislike very much, 2=dislike moderately, 3=neither like nor dislike, 4=like moderately, 5=like very much) to evaluate the acceptance level of several attributes, including taste, aroma, texture, and overall acceptance (Fibrianto et al., 2018). The formula with the highest acceptance level was then selected for the fortification with NaFeEDTA \cdot 3H₂O (416.57 mg/100 g, EMCE ferric-EDTA Ref-No-29265MC, Mühlenchemie GmbH & Co. KG, Ahrensburg, Germany) following the method of Jahan et al. (2019). The selected formula was packed and used for the intervention study.

Nutrient analysis: The nutrient analysis was conducted on both unfortified and fortified IBC3. Proximate analysis was performed following the method of Association of Official Analytical Chemists International (2005) to measure moisture (929.02), protein (975.17), fat (973.22), ash (922.02), and carbohydrate content (by-difference method). Energy content was calculated following the formula of Bassey et al. (2013) by multiplying the content of carbohydrates, protein, and fat by their respective energy value and taking the sum. All nutrients, except water, were determined as the dry basis. In addition, iron and zinc were determined using atomic absorption spectrometry (Doner and Ege, 2004).

Intervention of IBC

Subjects: Subjects were students from grades 1 to 3 of senior high school. The inclusion criteria were: (1) adolescent girl aged $16 \sim 19$ years; (2) having hemoglobin (Hb) levels of $8.0 \sim 11.5$ g/dL; (3) having no chronic disease, including liver disease, thalassemia, and hemophilia; (4) not a subject of other program addressing anemia; (5) willing to comply with all protocols of the study by signing the informed consent. A total of 50 subjects were screened and only 40 subjects were found suitable. This number met the minimum sample size according to Lemeshow et al. (1990) using 95% level of confidence and 80% power of the test. The subjects were assigned into two groups; subjects with moderate anemia (Hb lev $els=10.5 \sim 11.5 \text{ g/dL}$) were assigned to control group (n =20), and those with severe anemia (Hb levels= $8.5 \sim$ 10.4 g/dL) were assigned to intervention group (n=20). In addition, the subjects were in boarding school where the daily meal centrally provided by the school, thus variability in food consumption could be minimized.

The procedure of intervention: A week before the intervention, all subjects received deworming tablets (500 mg mebendazole) to eliminate the effect of worm infestation that impairs iron absorption (Samuel et al., 2013). The intervention group received fortified IBC3 (100 g) three times/week for 2 months replacing rice during lunch, while the control group received unfortified IBC3. Research assistant prepared IBCs 30 min before the lunch time and made sure that the subjects consumed the entire IBCs. Both fortified and unfortified IBC3 were similar in color, texture, and aroma so the subjects could not recognize the differences. All the study protocols have been approved by the Ethics Committee of IPB University

(No. 04/IT.3 KEPMSM-IPB/SK/2017).

Measurements: The measurements were carried out at the baseline and endline of intervention. The blood samples were collected in the morning by antecubital venipuncture. Subsequently, serum was collected from the blood samples by centrifugation (750 g, for 10 min) and stored in tubes under -20° C until use. The serum was used to estimate Hb and serum soluble transferrin receptor (sTfR) levels using the cyanmethemoglobin method (Drabkin reagent) and enzyme-linked immunosorbent assay (ELISA) kit (human sTfR ELISA, BioVendor, Brno, Czech Republic), respectively (Faber et al., 2005; Sazawal et al., 2010).

Data analysis

The acceptance level of all attributes between the formulas was tested using Kruskal-Wallis with Steel-Dwass test. A dependent *t*-test was used to analyze the changes in Hb and sTfR levels between pre-intervention and postintervention. For the differences between the control and intervention groups, an independent *t*-test was used.

RESULTS

Formulation and nutritional composition of selected IBC

IBC2 and IBC3 exhibited a hedonic score of more than 3 which is good enough to be accepted for all attributes, except the taste of IBC2 (Fig. 1). IBC3 (formula with longest soaking time) was selected as best performing in comparison to IBC1 and IBC2. IBC3 had a significantly higher acceptance level of taste and most acceptable texture than the other tested formulas. The aroma was not found to be significantly different between the tested formulas. However, the acceptance level was found to be



Fig. 1. Mean hedonic score of instant *Bose* formulas evaluated by 49 semi-trained panelists. Hedonic test used 5-point scale. Values are presented as mean \pm SD. Different letter (a-c) in the same attribute shows significant difference at *P*<0.05 using Kruskal-Wallis with Steel-Dwass post-hoc test. IBC, instant *Bose* corn.

positively related to the soaking time.

The nutritional composition of fortified and unfortified IBC3 was similar, except for the iron content (Table 1). The water content of fortified and unfortified IBC3 was 69.45% and 66.56%, respectively. The energy, ash, protein, fat, carbohydrate, and Zn content of fortified IBC3 were found to be 131.56 kcal/100 g, 3.45%, 9.49%, 8.90%, 78.13%, and 2.20 mg/100 g, respectively. In unfortified IBC3, the content of energy, ash, protein, fat, carbohydrate, and Zn were found to be 143.90%, 2.81%, 7.95%, 8.31%, 80.92%, and 1.65 mg/100 g, respectively. The fortification successfully increased the iron content of IBC3. The high content of iron (19.11 mg/100 g) was found in fortified IBC3 compared to unfortified IBC3 (2.65 mg/100 g). The iron content of fortified IBC3 could meet 127.4% of the Indonesian Recommended Dietary Allowance (RDA) of Iron for adolescent girls aged $13 \sim$ 18 years (15 mg/d) (Putri et al., 2022).

Effect of IBC3 on anemia status

Hb and sTfR levels of pre-intervention and post-intervention in fortified and unfortified IBC3 groups are shown in Table 2. Both IBCs significantly increased the Hb levels (P<0.05), but the increase was found to be significantly higher in intervention group (1.73 ± 1.21 g/dL) compared with control group (0.93 ± 0.72 g/dL, P=0.049). However, the effect of fortified IBC3 on sTfR levels was less significant. Unusually, the unfortified IBC3 demonstrated a significant increase in the sTfR (1.21 ± 0.93 ng/mL) levels.

DISCUSSION

The results suggest instant *Bose* has a shorter preparation time than the traditional method could be accepted according to the sensory evaluation. The results revealed soaking time of 20 min before cooking significantly improves the acceptance level. Soaking before preparation softens the texture of seeds increases water absorption which may increase the acceptability (Silva et al., 1981),

Parameter	Unfortified IBC3	Fe-fortified IBC3
Total energy (kcal/100 g)	143.90	131.56
Water content (%) ¹⁾	66.56	69.45
Protein (%)	7.95	9.49
Ash content (%)	2.81	3.45
Total fat (%)	8.31	8.90
Total carbohydrate (%)	80.92	78.13
Zn (mg/100 g)	1.65	2.20
Fe (mg/100 g)	2.65	19.11

¹⁾Determined as wet basis.

IBC, instant *Bose* corn.

Table 2, Effect of IBC on Hb and sTfR levels among adolescent

	Group		
Variable	Fe-Fortified IBC (n=20)	Unfortified IBC (n=20)	P-value ¹⁾
Hb (g/dL)			
Baseline	9.82±0.67	11.06±0.29	
Endline	11.55±1.50	11.99±0.81	
<i>P</i> -value ²⁾	<0.001*	<0.001*	
Changes	1.73±1.21	0.93±0.72	0.049#
sTfR (ng/mL)			
Baseline	34.86±18.09	18.84±8.02	
Endline	35.38±17.21	20.05±7.70	
<i>P</i> -value ²⁾	0.117	<0.001*	
Changes	0.52±1.12	1.21±0.93	0.087

Values are presented as mean±SD.

girls

¹⁾Between-group difference in change from baseline to endline analysis by independent t-test.

²⁾Within-group change from baseline to endline analysis by dependent t-test.

Significantly different from baseline to endline within the group at *P<0.05 and between the groups at *P<0.05.

IBC, instant Bose corn; sTfR, soluble transferrin receptor.

and decrease the soaking time (Abdel-Aleem et al., 2019). Increasing the soaking time improves the palatability by reducing the content of anti-nutrients such as phytate, tannin, oxalate, and saponin which are responsible for astringent taste (Adebayo, 2014; Petroski and Minich, 2020). Furthermore, soaking aids in the production of odor-active compounds which enhances the aroma of formulations (Zhang et al., 2018a). In addition, increasing the soaking time has a positive relationship with an increase in nutritional quality by increasing the bioavailability of beans and grains (Drumm et al., 1990; Adebayo, 2014; Abdel-Aleem et al., 2019). It has been shown that with longer soaking time in IBC increases porosity and rehydration and decreases hardness value (Eka Yulianti et al., 2022). However, a longer time of preparation may not be recommended as instant foods should be served quickly (Swamy et al., 2012).

In this study, IBC was consumed during lunch as a rice replacement. IBCs could at least provide 6%, 12%, 11%, 26%, and 18% of RDA for energy, protein, fat, carbohydrate, and zinc, respectively. IDA is commonly found to be coexisting with other nutrient deficiencies caused by poor dietary intake (Aspuru et al., 2011). A cohort study involving 93,676 participants by Thomson et al. (2011) found that besides having a low intake of iron, women with anemia had a lower intake of energy, protein, vitamin C, vitamin B12, folate, and red meat. It is estimated that people with caloric and protein restrictions have a high risk of anemia (Bianchi, 2016). Thus, IBC3s may provide not only iron but also additional overall nutrients. Fortified IBC3 could meet 100% of iron daily requirement while unfortified IBC3 can meet only 17.7%. Given that Indonesian adolescent girls' reported iron intake is less than 40% of RDA, consumption of foods high in iron is strongly advised (Kurniawan et al., 2006; Sumarlan et al., 2018; Rahfiludin et al., 2021).

Our intervention study demonstrated that both fortified and unfortified IBC3 significantly increased Hb levels of adolescent girls, however, the more pronounced increase was associated with fortified IBC3. Increasing iron intake has been widely accepted to increase Hb levels (Walter et al., 1993; Thuy et al., 2003; Beinner et al., 2005; Rivera et al., 2010). Also, a meta-analysis of 60 randomized controlled trials suggests that Fe-fortified foods increase Hb levels (0.42 mg/dL), and serum ferritin (1.36 μ g/L), and reduce the risk (RR) of anemia (RR= 0.59) (Gera et al., 2012).

Iron is an essential nutrient in Hb synthesis (Ginzburg, 2019). Hb is a molecule made up of four heme groups, a complex of ferrous iron with a porphyrin ring, and a globin group (Ogun et al., 2019). Low heme level due to low iron concentration reduces HRI protein leading to low production of some proteins including Hb (Zhang et al., 2018b).

Results of the present study exhibited that the severity of anemia is positively associated with the levels of sTfR. An increase in sTfR during anemia is due to a common mechanism by which cells need more iron for metabolism (Skikne, 2008). During anemia, sTfR continues to increase progressively as a cellular response to low iron (Yoon et al., 2015). However, sTfR was increased significantly in the control group while the intervention group showed no significant difference. On the other hand, previous studies demonstrated an inverse relationship between sTfR and Hb (Matsuda et al., 2002; Oustamanolakis et al., 2011; Skikne et al., 2011). The plausible explanation could be that IBC3 without fortification may not effectively improve the iron status of subjects. Results indicate that moderate anemia might progressively become severe if left untreated for at least 2 months. Additionally, the fortified IBC3 group's mean sTfR endline data was still borderline anemic, which may have been the reason why levels of sTfR did not fall in this group. Moreover, this study was conducted only for 2 months which may contribute to the absence of effects on the sTfR levels, while a previous study described sTfR reduction after 3 months of iron supplementation (Beguin, 2003). A meta-analysis showed that sTfR measurement is not a confirmatory diagnostic test, but only IDA screening due to relatively low sensitivity (86%) and specificity (75%) (Infusino et al., 2012). Additionally, the study's eight-week duration made it impossible to measure sTfR levels that had not yet achieved a steady state (Zhu and Haas, 1998).

In conclusion, the soaking time of 20 min in IBC had good acceptability according to sensory evaluation. The fortification of the instant *Bose* with NaFeEDTA·3H₂O significantly increased the iron content (127.4% RDA). After 2 months of intervention, both fortified and unfortified IBC3 notably increased Hb levels (1.73 ± 1.21 ; 0.93 ± 0.72) with more remarkable effect in the group of fortified IBC3. However, sTfR might not be sensitive to assess iron replenishment in this study. The results suggest that the fortified IBC could be an effective strategy to mitigate anemia among adolescent girls in Kupang, Indonesia.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: all authors. Analysis and interpretation: all authors. Data collection: LSG, ED, DM. Writing the article: all authors. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: LSG, SAM. Overall responsibility: all authors.

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