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# Research Article
# Evaluation of *In vitro* Iodine Absorption from Fortified Modified Cassava Flour (Mocaf)

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## Abstract
**Background and Objectives:** Mocaf reportedly contains a high content of amylose, which functions as an iodine carrier. Iodine is attached in a complex bond to its linear chain or to the branched chain of amylopectin. Evaluation of the absorption of iodine attached to amylose and amylopectin was performed to measure the amount of absorbed iodine in the small intestine. The absorption rate must be studied since iodine deficiency leads to health problems in all ages, especially affecting children's growth and development. This study was designed to evaluate *in vitro* iodine absorption of potassium iodate ($KIO_3$)-fortified mocaf in the small intestine. **Materials and Methods:** An *in vitro* evaluation of iodine absorption was conducted using the everted gut sac method. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). **Results:** The highest absorption occurred at 75 and 90 min, while the highest concentration was 40 ppm. The absorption rates at various concentrations were 56.23% (0 ppm), 65.53% (10 ppm), 69.29% (20 ppm), 71.91% (30 ppm) and 84.65% (40 ppm). The $KIO_3$ concentration significantly affected iodine absorption ($p \leq 0.05$). **Conclusion:** The highest absorption rate was 89.10%. A higher $KIO_3$ concentration increased iodine absorption and a longer absorption period tended to enhance the absorption rate.

Key words: Amylose, cassava flour, iodine deficiency, mocaf iodine absorption, potassium iodate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Iodine is one of the essential micronutrients needed for thyroid hormone synthesis and is important for growth and metabolism[1]. In fortification, iodine in $KIO_3$ form is often used[2] due to its favorable stability, even under humid storage conditions and longer shelf life[3].

Iodine deficiency might lead to health problems in all ages, including fetus, neonates, child, teenager and adult. Iodine deficiency can cause abortus, preterm birth, congenital anomaly, or cretin in the fetus and hypothyroidism and mental disorders in the neonates. In children and adolescents, iodine deficiency results in hypothyroidism, goiter, mental dysfunction and late physical growth, while adults might suffer from hypothyroidism, goiter with complications, or mental dysfunction[4]. Biofortification in food has often been used as an effective measure to prevent iodine deficiency-related health problems[5] and a strategy to reduce inadequate dietary micronutrient prevalence[6]. Fortification is also effective in reducing the prevalence of inadequate micronutrient intake[7].

Iodine bioavailability in food can be analyzed through absorption evaluation[8], *in vitro* studies[9,10]. The latter was reportedly suitable to measure iodine bioavailability in vegetable biofortification[11]. The absorbed iodine rate is related to the iodine content, chemical structure and the presence of goitrogenic factors such as iodine inhibitors[12].

Bioavailability represents the presence of a nutrient molecule in the blood circulation after absorption in the digestive track, starting from the duodenum and upper jejunum of the small intestine, with the highest absorption rate in the middle part of the jejunum[13]. Absorption in the small intestine occurs throughout the digestive tract but mostly in the jejunum[14].

To improve iodine intake through fortification, carriers need to be selected from widely consumed food in the Indonesian diet. Approximately 30.2% of the population consumes wheat flour and 19.5% consumes its processed products[15], despite import commodity. Thus, to overcome wheat dependency as well as food diversification with functional and economic benefits, fortification using a wheat flour substitute from local material such as modified cassava flour (mocaf) can be used as an alternative[16]. Cassava flour is modified through fermentation to generate a locally based product diversification alternative and to promote food security due to its high production potential as a main ingredient or as a substitution for wheat flour, rice/glutinous rice and as improver[17,18].

Mocaf also contains a high content of amylose, as shown by a study that demonstrated iodine-fortified mocaf (at 10-40 ppm) had 32.82-33.67% (db) amylose [19], which is an iodine carrier. Iodine is able to form complex bonds by attaching to the amylose linear chain and amylopectin branched chain[20]. Moreover, mocaf reportedly has 70.96-85.0% iodine retention at a $KIO_3$ level of 10-40 ppm after 3 months of storage[21].

The potential of iodine fortification was further studied through *in vitro* absorption evaluation of $KIO_3$-fortified mocaf in the small intestine. The iodine absorption rate was calculated based on the absorption period and $KIO_3$ concentrations.

This study aimed to measure iodine absorption of $KIO_3$-fortified mocaf in the small intestine using an *in vitro* everted gut sac method.

## MATERIALS AND METHODS

**Materials:** Experiments were conducted using potassium iodate ($KIO_3$)-fortified mocaf at concentrations of 0 (control), 10, 20, 30 and 40 ppm in an animal model of 2-month-old healthy male albino rats (*Sprague Dawley*) with an average weight of 180-200 g.

**Mocaf preparation and fortification:** Mocaf was obtained from a mocaf producer in Gunung Kidul district, Yogyakarta, Indonesia. Fortification using $KIO_3$ was performed in the Food and Nutrition Laboratory, Universitas Gadjah Mada (UGM). Fortified mocaf was maintained in storage tubes, ±20 g each, until absorption measurements based on potassium iodate concentration and period absorption.

**Methods:** Iodide transport against a concentration gradient by everted sacs of rat small intestines was studied in vitro[22]. Iodine absorption in vitro was evaluated using the everted gut sacs method. After 20-24 h of fasting and given only deionized water *ad libitum*, rats were anesthetized using ether before dissection of the abdominal cavity to remove the small intestine. One end of the intestine was tied and then gently everted using tweezers to prevent damage so that the villi were on the outside. The other end was connected to a small cannula using suture. Five centimeters of intestine was then filled with 1 mL serosal fluid consisting of 0.9% NaCl, similar to the physiological fluid of the mammalian body and subsequently placed in a fluid mucosal solution containing fortified mocaf. During experiments, the entire intestine section was kept immersed in mucosal fluid at 37°C,

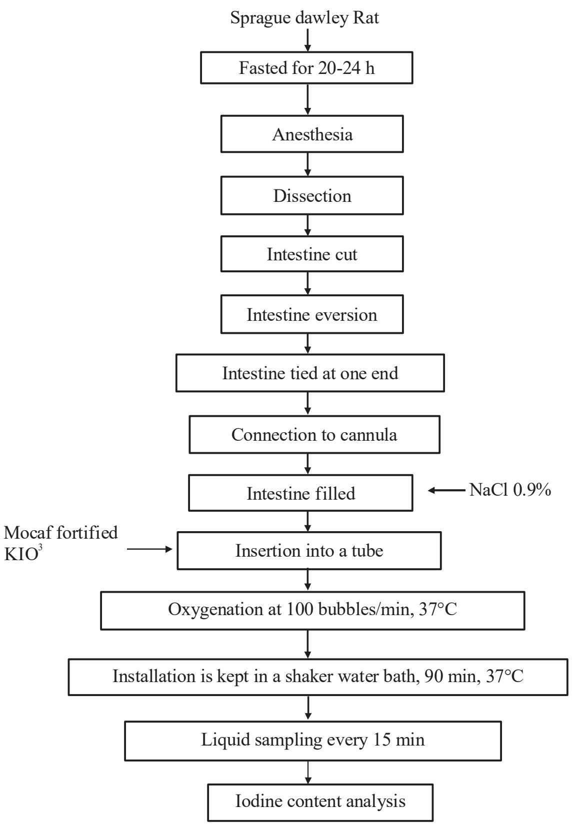


Fig. 1: Flow diagram of in vitro iodine absorption analysis (Modification Acland and Illman[22]; Murdiati[23])

oxygenated at 100 bubbles per minute and constantly stirred. Absorbed iodine was measured every 15 min for 90 min[23]. An *in vitro* iodine absorption flow is presented in Fig. 1.

**Statistical analysis:** One-way analysis of variance (ANOVA) was used to measure the treatment effect, followed by Duncan's Multiple Range Test (DMRT) when significant differences were found. Analyses were performed with SPSS 18.0 Statistical Software Program. Differences of $p<0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

**Iodine absorption based on the absorption period:** Absorption was measured to calculate the fortified iodine level absorbed in the gastrointestinal (GI) tract. The iodine absorption level of 0 ppm $KIO_3$ as a control increased up to 30 min, while the level at 10, 20, 30 and 40 ppm increased to 90 min, with the highest rate found between 75-90 min. The results indicated that a longer absorption period resulted in a higher iodine level. As the absorption period was cumulatively calculated, the iodine absorption rate increased (Fig. 2).

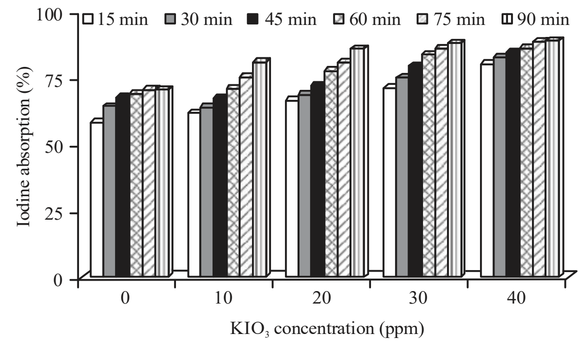


Fig. 2: Iodine absorption based on absorption period; $KIO_3$ at 0, 10, 20, 30 and 40 ppm during 15, 30, 45, 60, 75 and 90 min

An inadequate and imbalanced diet of goitrous patients might induce absorption disorders in the digestion system that inhibit Fe, iodine and thyroid function[24].

**Iodine absorption in various $KIO_3$ concentrations:** The value of iodine retention and absorption is needed to calculate iodine requirements in the prevention and effective control of iodine deficiency[25]. Iodine absorption was also measured in fortified processed products such as cereals, dairy and bakery products[26]. *In vitro* absorption is highly affected by the nutritional contents of those products[27]. The $KIO_3$ concentration significantly affected the absorbed iodine, with higher concentrations resulting in higher absorption ($p\leq 0.05$). This result was also reported by another study on the linear correlation between iodine intake concentrations and iodine content in cow milk[28]. Another report discussed iodine and its species in animal tissue in trace concentrations[29].

The results of this study found that the highest absorption rate of 89.10% was found at 40 ppm after a 90-minute period, while the average absorption value of each concentration at 0, 10, 20, 30 and 40 ppm were 66.72, 70.03, 75.30, 80.63 and 85.24%, respectively (Fig. 3).A higher $KIO_3$ concentration resulted in a higher absorption rate, with absorbed iodine depending on the iodine amount and form[12].Other research mentioned that 10.000 µg I $kg^{-1}$ dietary iodine in feed was high enough to affect sodium/iodide symporter (NIS) expression in the thyroid gland[30]. Other studies have reported that salt iodization has an absorption rate of 100%[31,32] and approximately 99%[31].

Serious efforts to eradicate IDD requires coordination from all stakeholders, effective monitoring of salt iodization, alternative fortification strategies and regional and local campaigning[33]. In addition, the findings must be able to encourage the food industry to use iodine fortification in products[25]. Micronutrients are needed for the process of

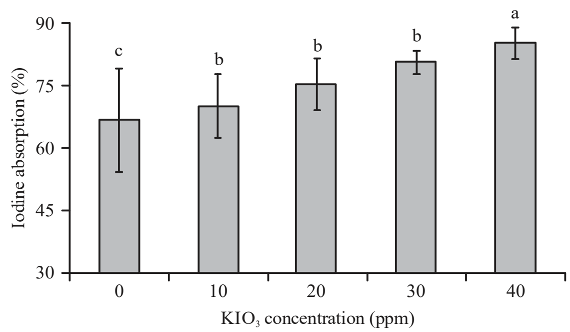


Fig. 3: Iodine absorption based on $KIO_3$ concentration at 0, 10, 20, 30 and 40 ppm

Different letters in different columns show significant differences ($p \leq 0.05$)

continuous construction and reconstruction. Micronutrient requirements differ depending on the needs of each individual related to metabolic conditions. Inadequate micronutrients have consequences for physical and cognitive development[34]. One of the micronutrients is iodine, which is an essential component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroid hormones regulate many important biochemical reactions, including protein synthesis and enzymatic activity and are critical determinants of metabolic activity[35]. The metabolism of iodine is illustrated as follows: Iodine is stored in the butterfly or H-shaped thyroid gland, which is located in the frontal part of the neck and consists of two lobes, the left and right parts, connected by the transverse isthmus in front of the trachea. Most iodine in the human body (70-80%) is supplied by the thyroid gland[36].

The biological plausibility of $KIO_3$-fortified mocaf is as follows: $KIO_3$ in mocaf is rapidly absorbed in the small intestine approximately 3-6 min after food digestion. Iodine enters the body in the form of an iodide ion ($I^-$) and is then rapidly absorbed through the gastrointestinal tract and distributed via extracellular fluid.

The process of iodide intake is initiated by the active transport system from capillaries into the glandular follicle cells. Iodide is taken into the thyroid gland by $Na^+$/symporter $I^-$ (NIS) located in the basolateral membrane of follicle cells. Iodide is oxidized in cells that move to the surface of the apical plasma membrane to enter the follicle lumen. Iodide is subsequently oxidized into iodine facilitated by thyroidal peroxidase (TPO).

Iodide ($I^-$), through iodination catalyzed by TPO together with hydrogen peroxidase ($H_2O_2$) bound to tyrosine in thyroglobulin (Tg), generates iodotyrosine precursor hormone (MIT) and diiodotyrosine (DIT). During the process catalyzed by TPO through coupling to form tyrosine (T4) and triiodotyrosine (T3) in the Tg of the follicle lumen, Tg enters via endocytosis and is digested, followed by T4 generating T3. Iodide is sent back to blood circulation by deiodination to be further utilized by the thyroid[37]. Iodine deficiency also affects Tg levels; specifically, abnormally high TSH levels are significantly correlated with serum Tg levels[38].

The acceptable daily intake of iodine in the potassium iodate form recommended by the World Health Organization (WHO) is 0.10-0.14 mg person$^{-1}$ day$^{-1}$ for adults[39], with a tolerable daily intake of 1 mg iodine day$^{-1}$ or 0.017 mg kg$^{-1}$ BB[40]; thus, iodine fortification using $KIO_3$ at 10-40 ppm in mocaf, as shown in the present study, meets the acceptable daily intake (ADI) standard. It is also safe without any potential harmful effect. Other studies recommend the dietary reference intake of 0.15 and 0.14 mg day$^{-1}$ for iodine has been established in the United States and Taiwan[41]. The addition of iodine at a higher amount than currently recommended must be considered[25].

**Implications:** This study was conducted to determine the safety and adequacy of the consumption of iodine needed by the body so that the addition of iodine can be performed correctly with several choices of $KIO_3$ concentration. Thus, this avoids the excessive use of $KIO_3$, deficiencies that can cause adverse effects on health or becoming less effective.

While the application of this research is a strategy to determine the amount of iodine absorbed in the small intestine, further information on iodine absorption values that need to meet iodine requirements is recommended. The results of our study can be applied in the community long term as daily consumption to improve nutritional quality and prevent iodine deficiency.

**Research recommendations:** The $KIO_3$ concentration used in this study was 10-40 ppm. The results showed that the concentration of 40 ppm showed the best results but this study recommends the use of $KIO_3$ concentrations as high as 10 ppm because at this concentration, $KIO_3$ has demonstrated the ability to achieve the iodine needs per person/day and is more economical compared to $KIO_3$ concentrations above (20-40 ppm).

**Limitations of the study:** There must be an increase in the number of samples in each research treatment to obtain results that can be more generalized. This study can also be followed by intervention research to determine the value of absorption of iodine consumed by the body.

## CONCLUSION

In this study, iodine was well absorbed in the small intestine, even though the iodine absorption was not 100%. This absorption is based on several $KIO_3$ concentrations and different absorption periods. The results of the study showed that the highest iodine absorption at 89.10% was obtained at 40 ppm $KIO_3$ after 90 min. A higher $KIO_3$ concentration resulted in higher iodine absorption and a longer absorption period tended to have higher $KIO_3$ absorption. Mocaf fortified with $KIO_3$ can be consumed in the form of processed snacks or wheat flour mixtures, can be consumed safely and does not cause health problems.

## SIGNIFICANCE STATEMENT

This study found a possible synergistic effect of $KIO_3$ concentration and duration of absorption period, which is useful for measuring the value of iodine intake absorbed by the small intestine of mice. The $KIO_3$ concentration and duration of absorption affect the absorption of iodine in the small intestine. The novelty of this study revealed the value of iodine intake absorbed in the small intestine in mice from $KIO_3$-fortified mocaf, which had not been explored by other researchers. Therefore, a new theory can be proposed regarding this combination of micronutrients and possibly food as other vehicles of iodine.

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