Moisture Effect on Characteristics of Slowly Digestible Potato Starch Prepared under Electron Beam Irradiation

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Received 3 October 2020; Revised 18 January 2021; Accepted 25 January 2021; Published 4 February 2021

This study evaluates the effect of initial moisture contents (11.74–29.84%) on physicochemical changes, in vitro and in vivo digestibilities of potato starch irradiated with electron beam (EB). After a constant dose of EB irradiation, intrinsic viscosity and average molecular weight of potato starch decreased for all investigated moisture contents. When the moisture of starch was lower than 18%, the depolymerization predominated, hence increasing the amylose content. At higher moisture, water can strongly absorb EB and produce highly active species that induced the crosslinking of amylose molecules and the disruption of large crystals into smaller defective crystals. As a result, we found a maximum in amylose content at 14.84% moisture and a minimum in the degree of crystallinity at 17.5% moisture. Thermal stabilities between the irradiated samples were not significantly different. In vitro digestibility results showed that higher moistures during EB treatment induced structural changes that led to the conversion of resistant starch (RS) fraction into slowly digestible starch (SDS). Moreover, an in vivo digestive model in mice showed that EB-treated starch was able to maintain blood glucose at a stable level for a long time. This study showed a potential for SDS production from potato starch using EB irradiation technology, even in large scale.

1. Introduction

Starch is the most common natural polymer and is widely used in many industrial applications, especially in food technology. Starch is the main nutritional ingredient in the human diet. Based on nutritional value, starch is divided into three fractions based on the rate of hydrolysis by digestive enzymes: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch [1]. RDS will cause a sudden increase in blood glucose level after ingestion. SDS is completely digested in the human small intestine, but its digestion rate is slower than that of RDS. SDS maintains blood glucose at a stable level for a long time and therefore plays an important role in a diet for people with type II diabetes. Besides, food products with high SDS content will prolong satiety and can be used by athletes to maintain a source of energy providing for the body for a long time. SDS can reduce the risk of cardiovascular disease and coronary heart disease. SDS has a positive effect on weight and obesity control. RS is the portion of starch that is not absorbed in the small intestine of healthy individuals [2, 3].

Various methods of starch modification and SDS production have been investigated based on chemical, enzymatic, physical, genetic, and combined treatments [2]. Electron beam (EB) irradiation is a “green technique” that has many advantages over others: no toxic chemicals used, no pollution, simplicity in operation, short time of treatment, and ease of scaling-up. The EB irradiation treatment can cause many changes in the starch such as the formation of free radicals, the formation of carbonyl/carboxyl groups, depolymerization, the formation of cross-linkages, changes in crystalline levels, and disruption of large crystals into smaller ones. These chemical and structural changes are susceptible to changes in physicochemical properties and starch digestibility [4]. There is not much research on the use of EB technology for making SDS-rich starch products [2].
To our best knowledge, there is no study evaluating the effect of initial moisture on the formation of SDS from potato starch. Therefore, this study will focus on investigating the physicochemical and digestive changes in potato starch with various initial moisture levels treated with electron beam irradiation.

2. Materials and Methods

2.1. EB Treatment of Starch. The initial moisture content of the native potato starch (Roquette Freres, France) in this study had a moisture content of 11.74% (CT11.74 sample). The starch samples (2.0 kg/sample) were adjusted to the desired moisture content (12–30%) by adding an appropriate amount of degassed distilled water, mixing well, and allowing to stabilize for 24 h at 30°C in a closed polyethylene bag. The actual moisture contents were determined again by a gravimetric method to ensure correct moisture values. EB11.74, EB14.84, EB17.51, EB19.24, and EB29.84 were the samples with actual moisture contents of 11.74, 14.84, 17.51, 19.24, and 29.84%, respectively. Each sample was then placed in two layers of vacuum-packed polyethylene bag and then treated with EB irradiation using a UELR-10-15S2 accelerator (Research and Development Center for Radiation Technology, Vietnam) on a conveyor belt running continuously with a dose of 5.5 kGy. The conveyor speed was controlled automatically to ensure the designed treating dose. The radiation dose was checked by a B3000 film dosimeter placed inside the starch sample before radiation. After treatment, the dosimeter plate was removed and dried at 60°C for 15 minutes to stabilize the colour. The absorbance of the dosimeter was measured at 1552 nm and compared to a conversion table to determine the treating dose. The treated sample was removed from the package and convectively dried (40°C) to achieve a final moisture content of 11.74% before storage in two layers of vacuum-packed polyethylene bag at 5°C for further experiments.

2.2. Intrinsic Viscosity and Molecular Weight (MW). The intrinsic viscosity ($\eta$) of the starch samples was measured according to a previous method [5, 6]. Potato starch was dispersed in a 1 M KOH solution to achieve a range of concentrations of 1–5 mg/mL. Starch solutions were thermally stabilized in a thermostatic bath at a temperature of 30°C. After that, an Ostwald capillary viscometer normally stabilized in a thermostatic bath at a temperature of 30°C. After that, an Ostwald capillary viscometer placed inside the starch sample before radiation. The radiation dose was checked by a B3000 film dosimeter placed inside the starch sample before radiation. After treatment, the dosimeter plate was removed and dried at 60°C for 15 minutes to stabilize the colour. The absorbance of the dosimeter was measured at 1552 nm and compared to a conversion table to determine the treating dose. The treated sample was removed from the package and convectively dried (40°C) to achieve a final moisture content of 11.74% before storage in two layers of vacuum-packed polyethylene bag at 5°C for further experiments.

2.3. Apparent Amylose Content. Apparent amylose content of starch was determined according to a method described in a previous study [7]. Starch (100 mg) was placed in a volumetric flask and wetted with ethanol (1 mL). Next, 1 M NaOH solution (10 mL) was added to dissolve the sample, shaken gently, and allowed to stand for 1 h until the solution was completely clear. Then, the flask was filled up with distilled water up to 100 mL. Two mL of this solution was transferred to another 100 mL volumetric flask, mixed with 50 mL of distilled water, and 2 drops of phenolphthalein indicator (1%, w/v in 95% ethanol). This solution was neutralized with 0.1 M HCl and then filled up to 100 mL with distilled water, and let stand for 30 min to stabilize the colour. The absorbance of the solution was measured in the range of 400–800 nm using a UV-Vis spectrophotometer (Lambda 25, PerkinElmer, Waltham, Massachusetts, USA). Apparent amylose content (%) of each starch sample was calculated according to the following formula:

\[
\text{% amylose} = \frac{A_{620} - A_{510} + 0.0203}{0.5002},
\]

where $A_{620}$ and $A_{510}$ were the absorbances of the sample at 620 and 510 nm, respectively.

2.4. X-Ray Diffraction and the Relative Degree of Crystallinity. X-ray diffraction (XRD) spectra of the starch samples were recorded using an X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany). The operating parameters were 40 kV and 40 mA with a radiation using Cu-K at a wavelength of 0.15406 nm and a scanning angle of 3–30° (2 theta) [8, 9]. The relative degree of crystallinity (DC) was determined by the following formula:

\[
\text{DC} = \frac{A_\alpha}{A_\alpha + A_a} \times 100,
\]

where $A_\alpha$ was the area of the crystal region and $A_a$ was the area of the amorphous region. The areas of these regions were calculated by Origin 8.5.1 software (OriginLab Corporation, Northampton, Mass, USA).

2.5. Pasting Properties. Pasting properties of potato starch sample were determined according to a published method [10]. A starch suspension (8%, w/w) was added to the Micro Visco-Amylo-Graph device (803203, Brabender GmbH & Co. KG, Germany), heated up from 30 to 93°C (7.5°C/min), and kept at 93°C for 15 min. Subsequently, the sample was cooled down to 30°C (7.5°C/min) and kept at 30°C for 15 min.

2.6. Morphological Property. The morphological characteristics of starch particles were observed on an SEM-S 4800 apparatus (Hitachi, Japan).

2.7. Thermogravimetry Analysis (TGA/DTG). The thermogravimetric measurements were performed with a TGA-60 apparatus (Shimadzu, Japan). Samples of about 10 mg were heated from 20 to 550°C with a heating rate of 10°C/min in a nitrogen atmosphere and with a nitrogen flow rate of 100 mL/min. The mass and the speed of mass change were plotted versus temperature as the TGA (thermogravimetric analysis) and DTG (differential thermal gravimetry) curves.
2.8. *In Vitro* Digestibility of Starch. *In vitro* starch digestibility was determined based on a published method [11]. Typically, pancreatin (1 g, P-7545, activity 8 × USP/g, Sigma-Aldrich, St. Louis, Mo., USA) was dissolved in distilled water (12 mL), stirred for 10 min, and then centrifuged at 2000 × g for 10 min. After that, 10 mL of the supernatant was mixed with 1.8 mL of distilled water and 0.2 mL of amyloglucosidase solution (AMG 300 L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark) and left in a 37°C water bath for at least 10 min. Each starch sample (30 mg) was placed in a 2 mL microtube containing a glass bead and 0.75 mL of sodium acetate buffer (pH 5.2). The tube was stored in a shaking incubator at 37°C and 240 rpm for 10 min. Then, 0.75 mL of the prepared enzyme solution was added to the tube, and the tube was shaken continuously. The reaction was stopped after predetermined periods by putting the tube in a boiling water bath for 10 min. The glucose concentration present in the supernatant obtained after centrifugation (5000 × g, 5 min) was measured using a GOD-POD kit (BCS, Anyang, Korea).

2.9. *In Vivo* Digestibility in Mice. *In vivo* digestibility was determined following the method in [12]. Swiss mice (*Mus musculus*, genuine female, 22.5 ± 2.5 g/individual, Ho Chi Minh City Pasteur Institute, Vietnam) were used for *in vivo* experiments. The sample size of *in vivo* experiments was calculated using the equation of a previous study [13]. Thirty-five mice were randomly divided into 7 groups (5 individuals/group). The mice were individually housed in an approved laboratory animal facility for 7-day adaptation period under controlled conditions (dark/light = 12 h/12 h, 30°C). The mice were fed with a standard food (Anifood, Institute of Vaccines and Biological Medical-IVAC, Vietnam) every 8 hours (3 times/day) and were free to drink sterilized water from a bottle. Before each experiment, the mice fasted for 16 hours. A 7.5% starch suspension (0.5 mL, w/v; gelatinized at 95°C for 30 min) or a 7.5% glucose solution (0.5 mL, w/v) was fed directly into the mouse stomach with an oral Zonde needle. Blood samples were taken from the tail vein of each mouse after feeding at 0, 30, 90, 120, 150, 180, and 240 min. Blood serum glucose levels were measured with an Accu-check Active (RDC_0007MM.V02, Germany). The blood glucose levels were evaluated by the areas under the blood glucose level curves, based on the procedure described in [14]. The postprandial energy was calculated based on estimation: 1 g of glucose = 4 calories. The *in vivo* experiments above were done under a certificate (No. IRB-A-2002, Institutional Review Board at Dinh Tien Hoang Institute of Medicine has the operating code as IRB-VN02010 issued by Vietnam Ministry of Health on 15th October 2015).

### 3. Results and Discussion

#### 3.1. XRD Pattern and the Relative Degree of Crystallinity

X-ray diffraction (XRD) is a useful measurement to determine the type of crystal and the degree of crystallinity (DC) in starch [15]. XRD patterns (Figure 1) show that, although there are some changes in intensity, position, and shape of the main peaks, all the starch samples still retained B-type crystal structures after EB treatment [16]. Results of DC calculations (Table 1) showed that low moisture contents (less than 18%) induced no statistically significant difference in DC of the starch samples on EB irradiation, while higher moisture content increased DC. This result highlighted the importance of water content on structural changes in starch under EB irradiation.

#### 3.2. Intrinsic Viscosity and Molecular Weight of Starch Samples

The frictional characteristics of starch solutions are influenced by the starch molecular properties and expressed through the intrinsic viscosity ($\eta_ι$) [6]. Increasing the initial moisture of starch resulted in a significant decrease in intrinsic viscosity and average molecular weight ($M_ω$) of starch after EB treatment (Table 1). Apparent amylose content (AM) of potato starch increased under the irradiation and reached the maximum value at an initial moisture content of 14.84%. However, if the moisture content was higher than 14.84%, the AM value decreased. Theoretically, there are two possible structural changes in starch caused by EB irradiation: (i) depolymerization which decreases the AM and intrinsic viscosity and (ii) crosslinks between amorphous amylose molecules that increase the DC [17]. When the moisture of starch was lower than 18%, the depolymerization predominated. Many other studies also found the depolymerization in EB-treated starches resulting in low-molecular-weight fractions [18, 19]. However, when the moisture of starch was higher than 18%, the crosslinking of amylose molecules predominates and results in lower amylose contents and higher degrees of crystallinity. This is because a high amount of water can increase the mobility of amylose molecules, as well as absorb EB irradiation to produce highly active species (OH, H, and $e^-_{eq}$), thus catalyzing the crosslinking reactions.

#### 3.3. Gelatinization Temperature

When heating a starch dispersion in water, the temperature at which the viscosity of the dispersion rises suddenly is called gelatinization temperature.
This is the minimum temperature required to cook a given starch. The viscosity peak appears at the balance between the expansion and the polymer leaching of starch granules. Peak viscosity and its temperature indicate the water-binding capacity of the starch sample [20, 21].

The irradiated starches with low moistures (<18%) have gelatinization temperatures (T_gel) lower than those of starches with high moistures (>18%) and not different from that of the native starch (Table 2). This result follows the DC tendency because starches with higher DC values tend to have higher gelatinization temperatures.

In general, all viscosities of the starch pasting profile (peak, trough, final, breakdown, and setback viscosities) of EB-treated starch samples were smaller than those of native starch. According to previous studies, this decrease is mainly related to the formation of free radicals and the depolymerization of starch molecules [17, 22].

Besides, the pasting profile viscosities of the treated starches increased with the increase in initial moisture content. The increase in cross-linkages between irradiated starch molecules can explain these changes [17]. Besides, an increase in the setback value indicated that the starch irradiated at high initial moisture content was more susceptible to the retrogradation.

### Table 1: Intrinsic viscosity, apparent amylose content, and molecular weight.

| Sample       | $\eta_i$     | MW    | AM  | DC     |
|--------------|--------------|-------|-----|--------|
| CT11.74      | 195.5 ± 2.2a | 128.2 ± 4.8b | 8.7 ± 0.1 | 22.7 ± 0.1a |
| EB11.74      | 124.4 ± 2.3c | 7.99 ± 0.14c | 11.2 ± 0.9c | 21.6 ± 0.2c |
| EB14.84      | 120.6 ± 1.2c | 7.71 ± 0.08 | 10.7 ± 0.2b | 20.2 ± 1.3c |
| EB17.51      | 117.5 ± 0.9b | 7.49 ± 0.06 | 8.3 ± 1.4c | 19.5 ± 2.7c |
| EB19.24      | 111.7 ± 1.7a | 7.07 ± 0.11 | 7.8 ± 0.4b | 29.6 ± 0.9d |
| EB28.94      | 128.2 ± 6.05 | 124.4 ± 0.8 | 10.7 ± 1.4 | 33.6 ± 1.3 | |

$\eta_i$: intrinsic viscosity (mL/g); MW (10^5 g/mol): average molecular weight; AM (%): amylose content; DC (%): degree of crystallinity. The results are expressed as mean ± standard deviation (n = 3). Numbers in a row with different superscript letters are significantly different (p < 0.05).

At the evaporation stage, there was no difference in mass losses between the starch samples. The peaks of the DTA curves of this stage were around 87°C.

DTA curves in the second stage show that both the native and the irradiated starch samples lost 50% of mass from 300 to 305°C. This suggested that, under the various treatment conditions in this study, the thermal stability of starch samples did not significantly change [24]. However, the DTA curves showed differences in the mass-loss rates of samples. Maximum mass-loss rates were in the following order: EB28.94 > CT11.74 > EB11.74 > EB14.84 > EB17.51 = EB19.24. An earlier study showed that the ratio of amylose/amylopectin affects the heat decomposition of starch [25]. Therefore, we found a similarity between the mass-loss rate, the amylose content, and the DC. The increase in crystallinity was correlated with a decrease in the mass-loss rate. This can be explained by the fact that the crosslinks formed during irradiation hindered starch oxidation.

At the third stage, there were no significant differences in mass losses between the starch samples. These thermogravimetric analysis results suggest that EB-treating starch with proper moisture contents can produce more heat-stable starch, compared to the native starch.

### 3.4. Morphological Properties.

SEM micrographs (Figure 2) show that the native starch and the EB-treated starch are irregularly oval with sizes ranging from 20 to 50 μm [23]. The difference in adhesion between the starch granules can be observed in the micrographs. Irradiated starches with a higher initial moisture demonstrated a higher adhesion. For example, EB28.94 sample had the highest cohesion compared to the others. Crosslink formation may explain this intergranule adhesion [17]. Similar results were reported in a study on the effect of gamma radiation on potato starch [22].

### 3.5. Thermogravimetric Properties.

TGA (thermogravimetric analysis) and DTG (differential thermal gravimetry) curves were used to examine the thermal stability of our starch samples (Figure 3). Typically, the mass loss of the starches occurs in three stages. The first stage is at low temperature (60–100°C) corresponding to the evaporation of adsorbed water. The second stage (100–300°C) corresponds to the heat decomposition of starch with water as the main product. The third stage (>300°C) is carbonization and ash formation [24].

### 3.6. In Vitro Digestibility.

The digestibility of starch attracts scientists due to its significant role in non-insulin-dependent diabetes treatments. The contents of RDS, SDS, and RS before and after treatment were determined for ungelatinized (a) and gelatinized (b) samples. The digestion fractions of ungelatinized samples are displayed in Figure 4(a). In general, there were not many differences in RDS fraction between samples. Increasing the initial moisture in EB treatment significantly increased SDS and decreased RS fractions. SDS and RS fractions of EB-irradiated starch samples were higher than those of the native starch. It seems that EB irradiation converted RS to SDS, and the presence of water enhanced this process.

After gelatinization (Figure 4(b)), RDS significantly increased. However, the correlation between SDS, initial moisture content, MW, and DC remained. EB19.24 contained the highest SDS level (47.2%). Especially, there is a balance between RDS and SDS fractions in treated starches with high moisture contents (EB17.51, EB19.24, and EB28.94).

Control of digestive hydrolysis on irradiated starch was reported [17]. The formation of linear chains and crosslinks under irradiation, which increases DC, can explain the
Table 2: Pasting properties of the starch samples.

| Characteristics     | CT11.74 | EB11.74 | EB14.84 | EB17.51 | EB19.24 | EB28.94 |
|---------------------|---------|---------|---------|---------|---------|---------|
| $T_{gel}$ (°C)      | 60.9 ± 0.2$^a$ | 61.1 ± 0.0$^{bc}$ | 61.0 ± 0.1$^{ab}$ | 61.2 ± 0.1$^c$ | 61.4 ± 0.1$^d$ | 62.7 ± 0.1$^e$ |
| $V_{peak}$ (BU)     | 1482 ± 1$^f$ | 699 ± 3$^a$ | 833 ± 8$^b$ | 891 ± 1$^c$ | 961 ± 3$^d$ | 1195 ± 5$^e$ |
| $V_{trough}$ (BU)   | 385 ± 2$^f$ | 73 ± 1$^a$ | 89 ± 1$^b$ | 98 ± 1$^c$ | 123 ± 3$^d$ | 400 ± 2$^e$ |
| $V_{final}$ (BU)    | 1122 ± 4$^f$ | 211 ± 1$^a$ | 218 ± 1$^b$ | 235 ± 1$^c$ | 255 ± 3$^d$ | 707 ± 1$^e$ |
| $V_{breakdown}$ (BU)| 1098 ± 6$^f$ | 626 ± 7$^a$ | 744 ± 2$^b$ | 793 ± 3$^c$ | 838 ± 1$^d$ | 795 ± 1$^e$ |
| $V_{setback}$ (BU)  | 559 ± 2$^f$ | 77 ± 3$^a$ | 80 ± 1$^b$ | 85 ± 1$^c$ | 91 ± 5$^d$ | 329 ± 3$^e$ |

The results are expressed as mean ± standard deviation (n = 3). Means in a row with different superscript letters are significantly different ($p < 0.05$).

Figure 2: Scanning electron micrographs of starch samples: (a) CT11.74; (b) EB11.74; (c) EB14.84; (d) EB17.51; (e) EB19.24; (f) EB28.94.

Figure 3: TGA-DTA curves of starch samples.
increase in RS fraction [26]. However, in our study, RS was converted to SDS. At low moisture contents (11.74 to 14.84%), the MW and DC values decreased, while the RS was converted to SDS. These changes might be an indication that the crystalline area in the starch was broken into smaller amorphous areas [3, 27]. Besides, at moisture contents higher than 14.84%, the MW decreased while the DC value increased significantly, while the RS shifted to the SDS. This phenomenon reflected the formation of new crystalline regions. Because of the limitation of the chains, crosslinking of linear chains products from depolymerization results in less perfect crystals [3, 27]. These nonperfect crystals were the reason for the significant increase in SDS fraction. This increase in SDS content implies many health benefits such as stabilizing glucose metabolism, limiting diabetes, and feeling full [17].

3.7. In Vivo Digestibility. In vivo digestive assay in mice is a common method to determine the effects of starch on living organisms [12, 28]. Figure 5 shows the blood glucose responses in mice after feeding with glucose or starch samples. The blood glucose level curves of the samples in this study were similar to those in the previous studies [12, 29]. The preprandial blood glucose level in mice was around 70–73 mg/dl. It was reported that RS is almost unrelated to blood glucose level [12]. The postprandial blood glucose levels of glucose and amorphous starch reached the maximum after 30 minutes of feeding. Samples with high SDS content showed high blood glucose levels at the 90th minute. After the 150th minute, the blood glucose was rather low (<85 mg/dl). In this study, we divided the blood glucose curve (the in vivo digestibility) in mice into three stages (Figure 5 and Table 3): (I) fast digestion (0–60 minutes), (II) slow digestion (60–150 minutes), and (III) indigestion (>150th minute).

The total obtained energy (calories), the obtained energy of each stage, and the glyemic index (GI) of the starch samples are shown in Table 3. The postprandial energy of glucose sample was the highest, and the lowest belonged to the native starch (CT11.74). The total energy and the GI of EB samples were higher than that of the native starch. These parameters were ranked as follows: glucose > EB14.84 = EB17.51 > EB19.24 > EB11.74 > EB28.94 > CT11.74. Previous studies showed that irradiation treatment could alter starch digestibility [17]. In this study, the depolymerization, the disruption of large crystals, and the formation of nonperfect crystals may...
cause the irradiated starch samples more susceptible to digestive enzymes than the native starch.

At stage I (0–60 min), the blood glucose curves reached the maxima at the 30th minute, except for EB17.51 (60th minute). For the glucose sample, within 30 minutes after feeding, the blood glucose response in mice increased sharply (3.6 times higher than before feeding) but then decreased quickly. Although not as high and fast as the glucose, CT11.74 sample also caused the blood glucose response in mice to increase and then decrease rapidly. During 30–60 minutes after feeding, the blood glucose level induced by the EB-irradiated starches decreased more slowly than that of the native starch. At stage II (60–150 minutes), the blood glucose response of mice fed with glucose continued to decline rapidly. At the same time, the blood glucose levels of mice fed with the EB-irradiated starches were higher than those fed with the native starch and the glucose. There is a correlation between the % energy and SDS content of samples: a high SDS content results in a high % energy gained at this stage, and vice versa. Furthermore, the blood glucose level of EB19.24 and EB28.94 samples decreased more slowly than the others. The results showed that the digestive fractions of samples of in vivo and in vitro measurements were similar (Figure 4 and Table 3). The energy gained at this stage of EB19.24 was the highest, corresponding to the highest SDS content. According to Lee et al. (2012), these samples were a sustainable source of energy. The energy balance between stage I and stage II indicates that these samples can sustain stable blood glucose levels in animals.

4. Conclusions

EB irradiation can be used as a technique to modify potato starch. EB irradiation on starches with different initial moisture levels has marked effects on the structural, physicochemical, and digestive properties of starch. This research demonstrates a new technique that converts RS into SDS, thereby changing the nutritional value of the product. Due to the ability to be performed continuously on a large scale, this technique of starch modification can have a high impact.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

All authors contributed equally to this study.

Acknowledgments

The authors thank Ho Chi Minh City University of Technology and Education for financial support, facilities, and equipment supports to complete this study. The authors gratefully acknowledge Ms. Chau Thi My Thuyen for helpful technical assistance.

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