Contents lists available at Science-Gate

International Journal of Advanced and Applied Sciences

Journal homepage: http://www.science-gate.com/IJAAS.html

Electron beam irradiated maize starch: Changes in structural, physico-chemical properties, and digestibility

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ARTICLE INFO

Article history:
Received 8 October 2019
Received in revised form
5 January 2020
Accepted 8 January 2020

Keywords:
Digestibility
Electron beam
FTIR
Maize starch
Molecular weight
X-ray diffraction

ABSTRACT

Electron beam (EB) irradiation was applied on maize starch at doses from 0 to 7 kGy. Electron beam irradiation resulted in (a) depolymerization of starch molecules, (b) formation of cross-linkages leading to the increasing of crystalline regions, and (c) degradation of the large crystalline region to smaller one. The increasing of irradiation dose resulted in the reduction of pH, reduced viscosity, molecular weight, and the increase of free acid content. During treatment, the color of the sample became darker. FITR spectra, A-type X-ray diffraction pattern and the ratio of amorphous/alpha-helix region of starches were not significantly changed during treatment. These mechanisms above caused not only the changes in amylose content but also the reduction of swelling factor and the increase of solubility. Furthermore, the interaction of these mechanisms could be explained to the conversion between slowly digestible starch and resistant starch fractions.

1. Introduction

Starch is one of the natural polymers that is widely applied in industries, especially in pharmaceuticals and food industries. Although cheap in cost and availability, the characterization of raw starch displays some disadvantages in applications. Thus, raw starch should be modified by various techniques to change its structure, functional properties, and digestibility. Therefore, starch is usually subjected to different treatments (physical, chemical and enzymatic treatments) to modify its properties resulting in desired properties suitable in various industrial applications. Theoretically, starch could be modified using physical, chemical and enzyme treatments. Irradiation, a physical modification, is a "green", simple, fast, low cost and eco-friendly treatment. High-energy irradiated-materials are possible applications for various industrial fields. Under irradiation, like an electron beam, starch can be modified and changes in its properties. The nature of modification in starch by electron beam (EB) irradiation depends on various factors such as dose of irradiation, nature of irradiation, source of starches. Irradiation could be an effective treatment for starch modification through cross-linking, grafting and degradation reactions (Pimpa et al., 2007; Braşoveanu and Nemţanu, 2018). Base on determining factors (dose, moisture, rate) of EB treatment, most of the previous studies reported the effect of radicals on starch, resulting in changes in structure, physico-chemical properties and digestibility. 10 kGy-irradiation of maize and kidney bean flours resulted in a decrease in pasting viscosity (Rombo et al., 2001). Pimpa et al. (2007) reported a decrease in swelling power following an increase of irradiation dose. Furthermore, irradiation changed starch digestibility in either cultivar (Polesi et al., 2018). In this study, we objected to investigate the effect of dose of EB irradiation on structural, physico-chemical properties and in vitro digestibility of maize starch.

2. Material and methods

2.1. Irradiated starch

Maize starch (Roquette Riddhi Siddhi, India, moisture content of 7.74%) was sealed in polyethylene bags. Then, the sample was continuously irradiated using UELR-10-15S2 equipment (CORAD Services Ltd, St. Petersburg, Russia) at the speed of 2 kGy/min in a conveyor. EB7, EB5, EB2.7, EB1, EB0 were irradiated starch at 7.0, 5.0, 2.7, 1.0 and 0 kGy, respectively.

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2.2. Free acidity

Free acidity was determined by a method of Sokhey and Chinnaswamy (1993). Starch (1.0 g) was suspended in degassed, boiled distilled water with continuously shaking for 10 min. This suspension was titrated by 0.023 N standardized NaOH solution.

2.3. Color space

The color of the sample was measured by a Minolta-CR400 (Japan). The difference of color (ΔE) was calculated as (Mokrzycki and Tatol, 2011):

$$\Delta E = \sqrt{(L_i + L_o)^2 + (a_i + a_o)^2 + (b_i + b_o)^2} \quad (1)$$

In this, L_o, a_o, and b_o were collected from EB0 (raw starch); L_i, a_i, b_i were collected from irradiated samples.

2.4. Solubility and swelling power

Solubility (SB) and swelling power (SP) of samples were examined following the method of Schoch (1964). Starch (2.0 g) was put into a pre-weighted centrifugal tube. Distilled water was added into this tube to give a final weight of 180 g. Then, the starch slurry was completely suspended and was immediately placed in 85°C with continuous shaking. After 30 min, distilled water was added to reach the final weight of 200 g. The tube was centrifuged (1000×g, 15 min). For measuring solubility, the supernatant (50 ml) was pipetted and transferred into an evaporating petri dish and dried 105°C for 24 h. For measuring swelling power, the supernatant was carefully removed and discarded. The tube with sediment paste was weighted.

$$\text{Solubility (\%)} = \frac{\text{weight of soluble starch}}{\text{weight of sample on dry basis}} \times 400 \quad (2)$$

$$\text{Swelling power} = \frac{\text{weight of sediment paste} \times 100}{\text{weight of on dry basis}} \times (100 - \text{solubility}) \quad (3)$$

2.5. Viscosity and average molecular weight

Intrinsic viscosity (η_i, ml/g), which was dissolved in a basic solution, related to structure, size, shape and molecular weight of starch molecules. Thus, base on intrinsic viscosity, the average molecular weight (M_w) of starch could be estimated. A series of starch solution (1.0 to 6.0 mg/ml in 1 M KOH) was prepared (Dokic et al., 2004; Harding, 1997). These solutions were determined their kinematic viscosity (η, m^2/s) using an Oswald viscometer (Ø=0.3 mm, Ref. No 509 03, Germany). Starch solutions were kept in 30°C for 30 min before measurement. Distilled water was used as a standard. Kinematic viscosity was calculated following an equation (2): $\eta = 0.004 \times t - \frac{0.12}{t}$ (www.si-analytics.com). Density (ρ) of starch solutions was determined using an equation (3) $\rho = \frac{m}{V}$; m was weight of sample (g), V was volume of sample (ml). Relative viscosity (η_{rel}) was calculated using an equation (4): $\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o} \times \frac{\rho}{\rho_o}$; η_o was kinematic viscosity of distilled water (m^2/s), t was flowing time (s) of the solution in viscometer, t_o was flowing time (s) of water in viscometer, ρ was the density of the sample at 30°C, và ρ_o was the density of water at 30°C. Reduced viscosity (η_{red}, ml/g) was measured following an equation (5): $\eta_{red} = \frac{\eta_{rel} - 1}{c}$; c was a concentration of maltodextrin. Intrinsic viscosity (η_i, ml/g) of starch samples was determined using an equation (6): $\eta_i = \lim_{c \to 0} \eta_{red}$. Following Mark-Houwink equation (7), the average molecular weight of the sample as $\eta_i = K M_w^a$. Base on, $K = 1.18 \times 10^{-5}$; a=0.89; M_w (g/mol) was the average starch molecular weight (Cowie, 1960).

2.6. Iodine binding property and apparent amylose content

The iodine binding property was measured following the method of Zhu et al. (2008). The absorbance of the starch-iodine complex was scanned from 400 to 800 nm. Apparent amylose content (AM, %) was calculated using an equation:

$$AM = \frac{Abs_{620nm} - Abs_{510nm} + 0.0542}{0.3995} \quad (4)$$

2.7. FTIR spectra

FTIR spectra of samples were scanned from 400 to 4000 cm^{-1} (Kizil et al., 2002) using an FTIR device (FTIR-8400S, Shimadzu, Japan). KBr (0.2 g) and starch (2.0 mg) were mixed well and ground. Then, the mixture was pressed at 8.0 bar for 10 min. The pressed sample was used for FTIR spectra scanning.

2.8. X-ray diffraction pattern and degree of relative crystallinity

XRD was determined using a powder X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany). The operating conditions were 40 kV and 40 mA with Cu-Kα radiation of 0.15406 nm (Nickel filter; time constant, 4 s). Each scan was performed from 3 to 30° (2θ). DRC was calculated using the equation $DRC = \frac{A_c + A_a}{A_c}$, where A_c is the area of the crystalline portion and A_a is the area of amorphous portion, according to the method of Nara and Komiya (1983) with peak-fitting software (Origin version 7.5, OriginLab, Northampton, Mass., U.S.A.). The amorphous sample was prepared autoclaving starch slurry (2.0 %) at 121°C for 30 min. Then, autoclaved starch was freeze-dried.

2.9. In vitro digestibility

Starch digestibility was determined according to the method of Englyst et al. (1992), with a slight modification of Shin et al. (2007). Pancreatin (2.0 g, Sigma–Aldrich) was dissolved in distilled water (24 ml) and stirred for 10 min. It was centrifuged (1500

×*g*, 10 min). Then, the supernatant (20 ml) was mixed with distilled water (3.6 ml) and amyloglucosidase (0.4 ml, AMG 300L, Novozymes). The starch sample (30 mg) was placed in a 2-ml microtube with a glass bead. After adding sodium acetate buffer (0.75 ml, pH 5.2), the tube was shaken continuously (37°C, 10 min, 240 rpm). Subsequently, the prepared enzyme solution (0.75 ml) was added to the tube. The reaction was stopped after 10 or 240 min by boiling for 10 min. The glucose present in the supernatant obtained by centrifugation (5000 ×*g*, 5 min) was measured using a GOD-POD kit (BCS, Anyang, Korea). Starch fractions were classified based on the rate of hydrolysis. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured by the glucose concentration after enzyme reaction for 10 and 240 min, respectively. Resistant starch (RS) constituted the fraction undigested after 240 min.

2.10. Statistical calculation

All values were shown in the average ± standard deviation (n=3). The data were calculated differently by one-dimensional Anova calculation ($P<0.05$, Duncan's multiple range test) using SPSS software (Released 2008, SPSS Statistics for Windows, Version 17.0, Chicago: SPSS Inc.).

3. Results and discussion

3.1. Free acid and apparent amylose contents

The increasing the irradiation dose caused a decrease in the pH of the sample, while it caused the decrease of free acid content (FA) (Table 1). Obviously, under electron beam irradiation, the degradation of starch molecules resulted in the formation of –COOH groups (Pimpa et al., 2007). Moreover, the cleavage of side-chains out of amylopectin produced linear starch molecules. Consequently, the formation of linear starch molecule-iodine complex increased resulting in the increase of amylose content (AM) (Table 1). The further degradation, under a higher dose of treatment, caused the shorten of linear molecules to shorter ones. During the degradation of linear amylose, the color of the complex changed from violet-blue (DP 39-40) to brown (DP 21-24). If DP<20, linear molecules could not bind to iodine (Cui, 2005). This mechanism could explain to the reduction of AM at irradiation dose ≥5.0 kGy compared to its at lower doses. Fig. 1 shows reduced viscosity (η_{red}) of samples at various irradiation doses.

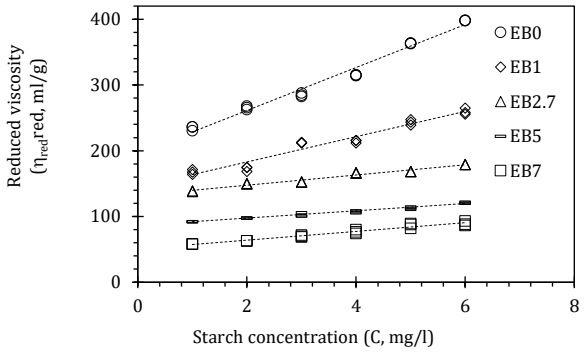

Fig. 1: Reduced viscosity (η_{red}) of samples at various irradiation doses

3.2. Viscosity and average molecular weight

Reduced viscosity (η_{red}) of the starch sample negatively correlated with EB irradiation dose (Fig. 1). Besides, the higher the concentration of the starch solution was, the higher η_{red} value was. Intrinsic viscosity (η_i) was calculated from both the equation (6) and the trendline ($R^2>0.95$) of reduced viscosity (Table 1). The increase of irradiation dose (from 0 to 7.0 kGy) resulted in a decrease of both η_i and average molecular weight (M_w) of starches. Actually, these characteristics were pieces of evidence of the degradation of starch molecules to smaller fractions (Pimpa et al., 2007).

Table 1: Properties of EB irradiated starches

Properties	Starch samples				
	EB0	EB1	EB2.7	EB5	EB7
pH	6.7 ± 0.0^{a}	6.5 ± 0.0^{b}	6.2 ± 0.0^{c}	5.8 ± 0.1^{d}	5.4 ± 0.0^{e}
FA	9.58 ± 0.66^{e}	11.50 ± 0.00^{d}	12.65 ± 0.00^{c}	4.57 ± 0.66^{b}	16.87 ± 0.66^{a}
AM	24.3 ± 0.2^{b}	24.4 ± 0.2^{b}	28.9 ± 0.3^{a}	24.6 ± 0.2^{b}	21.9 ± 0.3^{c}
a*	32.666	19.233	7.7616	5.5294	6.6464
b* (η_i)	195.89^{a}	144.47^{b}	132.05^{c}	86.558^{d}	50.723^{e}
R^2	0.9821	0.9515	0.9703	0.9704	0.958
M_w	7.33^{a}	5.21^{b}	4.71^{c}	2.93^{d}	1.61^{e}
DRC	26.29 ± 1.16^{e}	28.22 ± 1.15^{d}	32.26 ± 0.27^{c}	34.66 ± 0.57^{b}	38.96 ± 0.08^{a}
SB (%)	7.2 ± 0.8^{d}	10.4 ± 0.8^{c}	12.4 ± 1.4^{bc}	13.1 ± 2.6^{b}	22.0 ± 1.6^{a}
SP	10.8 ± 1.4^{a}	9.7 ± 0.7^{b}	8.6 ± 0.2^{c}	7.4 ± 1.1^{d}	6.9 ± 1.2^{e}
L	98.29 ± 1.2^{a}	98.26 ± 0.5^{a}	98.05 ± 1.0^{a}	97.94 ± 2.1^{a}	97.65 ± 2.0^{a}
a	-2.45 ± 0.00^{a}	-2.36 ± 0.01^{a}	-2.29 ± 0.01^{a}	-2.28 ± 0.00^{a}	-2.27 ± 0.00^{a}
b	4.74 ± 0.02^{a}	4.92 ± 0.00^{a}	5.16 ± 0.00^{b}	5.56 ± 0.01^{ab}	6.03 ± 0.02^{b}
ΔE	0.00 ± 0.00^{a}	0.20 ± 0.01^{b}	0.50 ± 0.00^{c}	0.91 ± 0.01^{d}	1.45 ±0.00^{e}
RDS	2.45 ± 0.36^{b}	2.55 ± 0.20^{b}	2.70 ± 0.32^{b}	2.58 ± 0.13^{b}	3.47 ± 0.41^{a}
SDS	64.57 ± 5.42^{b}	55.65 ± 2.34^{c}	64.33 ± 3.58^{b}	68.45 ± 5.34^{b}	74.01 ± 4.78^{a}
RS	32.97 ± 5.42^{b}	41.79 ± 2.34^{a}	33.08 ± 3.58^{b}	28.85 ± 5.34^{b}	22.52 ± 4.78^{c}

FA (ml): Free acid content; AM (%): apparent amylose content; a, b*: Factors of trendline equation (y=a*x+b*) in Fig. 1; R^2: R square factor of trendline; M_w (×10^5 g/mol): Average molecular weight taken from equation (7); η_i: Intrinsic viscosity; DRC: Ratio (%) crystalline region; SB (%): Solubility; SP (%): Swelling power; L, a, b: Color white/black, red/green, yellow/blue, respectively; ΔE: The color difference of samples comparing to EB0; WI: Water index; RDS: Fraction (%) of rapidly digestible starch; SDS: Fraction (%) of slowly digestible starch; RS: Fraction (%) of resistant starch. All values were shown in the average (± standard deviation). Superscripts in each row indicate the significant differences (P<0.05)*

3.3. FTIR spectra

FTIR was a useful tool for the characterization of chemical changes in starch. Fig. 2 showed no significant difference in the spectra of starches; the appearance or disappearance of peaks of treated starch was not detected compared to those of EB0 (raw starch). It indicated there was not any formation or loss of chemical groups in samples under EB irradiation. However, the absorbances of peaks were different reflecting the quantitative chance of chemical structure. FTIR spectra of irradiated starches were divided to four main regions that could successive interpretation and characterization of the key bands (Kizil et al., 2002): (i) <800 cm^{-1}, (ii) 800-1500 cm^{-1} (the fingerprint region), (iii) 2800-3000 cm^{-1} (C-H stretch region), (iv) 3000-3600 cm^{-1} (O-H stretch region).

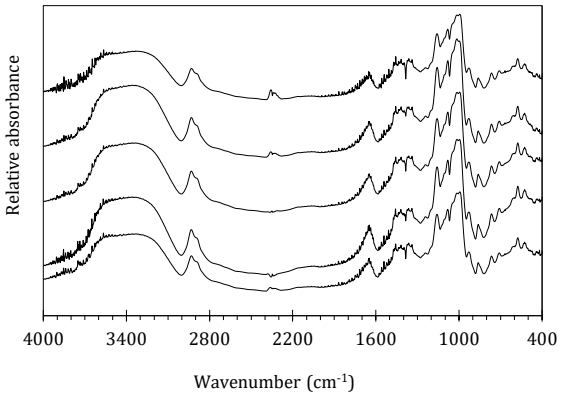

Fig. 2: FTIR spectra of irradiated starches (from the top to the bottom: EB7, EB5, EB2.7, EB1, EB0)

The first (i) region exhibited complex vibrations caused by skeletal mod vibrations of the glucose pyranose ring. In the second (ii) region, band at 936 cm^{-1} was the evidence of alpha-1, 4 glycosidic linkages in starch. Theoretically, the region 900-950 cm^{-1} reflected glycosidic-linkage-related vibration modes (C_1-O and C_4-O). Glucose in aqueous conditions was irradiated to destructure the pyranose ring and produce some radicals. However, this destruction could not be detected in starch or other polysaccharides. The absorbance of the peak at 1047 cm^{-1} was sensitive to the amount of ordered region and the absorbance of 1022 cm^{-1} and 1035 cm^{-1} were the measurement of amorphous starch. Thus, the ratio (R) of the height of the bands at 1047/1022 cm^{-1} or 1047/1035 cm^{-1} expressed the amount of ordered starch (alpha helix) to amorphous starch (van Soest, 1995). In this study, R values were found in ranges of 0.93-0.94 and 0.97-0.98 base on two above calculations, respectively. Thus, EB irradiation did not significantly affect the ratio of ordered/amorphous structures of starch. Brasoveanu et al. (2013) stated, during EB irradiation, large crystals might be broken into smaller ones resulting in the significantly unchanged R-value. Similar behavior was seen in gamma-irradiated cowpea at doses lower than 50 kGy. The third region (iii) was used to detect irradiated starch. Water was radicalized to form radical $^{\bullet}OH$ that attack C-H bonds to release hydrogen atom. This reaction was preferred in aqueous starch. In Fig. 2, the third region did not any difference between samples. Based on the results of the FTIR spectra of the last region (iv), researchers reported there was not an excessive amount of free radicals (hydrogen atom, solvated radiolysis and hydroxyl radical) produced during the radiolysis of bound moisture. If the insufficient amount of free radicals was formed, the radiation-induced reaction in starch macrostructure could not be significant (Kizil et al., 2002). In this study, there were insignificant changes in spectra or height of peaks. Actually, in the non-aqueous condition of starch, changes in FTIR results could not be easy to be detected (Kizil et al., 2002).

3.4. X-ray diffraction pattern and relative crystallinity

X-ray diffraction pattern (XRD) of the samples exhibited an insignificant change in crystal type under EB irradiation (Fig. 3). There was not any formation or disappearance of peaks. Typically, peaks at 15.2°, 17.2°, 18°, 20.2°, 23° (2 θ) were characteristic for the A-type X-ray pattern (Cui, 2005). A previous study reported XRD of gamma-irradiated maize starch does not change under a dose of 500 kGy (Liu et al., 2012). Kong et al. (2016) stated the irradiation dose lower than 7 kGy could result in an increase in the degree of relative crystallinity (DRC). In contrast, the dose of >9 kGy caused a decrease in DRC. Researchers found that the formation of cross-linkages in starch molecules leading to this increase (Brașoveanu and Nemțanu, 2018). Other researchers reported dose up to 50 kGy did not affect on DRC. In this study, relative crystallinity (DRC) of starch increase following the increase of EB dose (Table 1). Thus, the change in DRC might be affected not only by irradiation dose but also by other factors.

3.5. Color difference

The color of the samples was slightly changed during the EB irradiation (Table 1). The value of a and b was slightly increased, indicating irradiated starches became redder and more yellow than raw starch. However, the reduction of L value reflected the darker in color of treated starches. Actually, similar results were found in EB irradiated sago starch (Pimpa et al., 2007). Kang et al. (1999) reported an insignificant change in value whilst increased b value was found under gamma irradiation of maize starch. Thus, the difference in color under ionized irradiation base on desired treatment and starch sources. The changes in color could be caused by caramelization of monosaccharides, which were degraded from starch molecules under irradiation (Greenwood and Mackenzie, 1963). Furthermore, the differences in

color (ΔE<1.0) of EB1, EB2.7 and EB5 showed that they cannot be recognized by human eyes. On the opposite, at 7.0 kGy, the difference was 1<ΔE<2 reflecting it was just being found by an experienced examiner (Mokrzycki and Tatol, 2011).

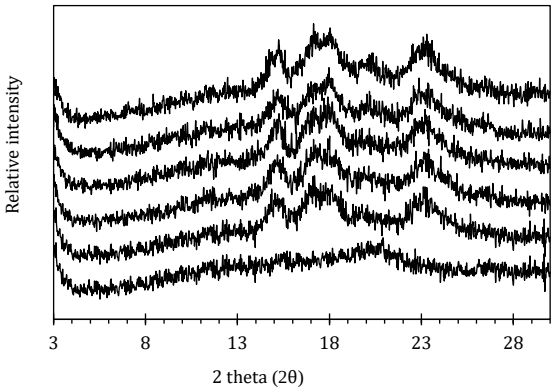

Fig. 3: X-ray diffraction pattern of samples (From the top to the bottom: EB7, EB5, EB2.7, EB1, EB0 and amorphous)

3.6. Solubility and swelling power

The higher the irradiation dose is, the higher the solubility and the lower swelling power is (Table 1). As the dose was increased, the swelling power (SP) and solubility (SB) of irradiated starches respectively decreased and increased. Theoretically, the swelling begun at amorphous and intercrystalline regions of starch granules leading a tension on neighboring crystals and tends to distort them. Further heating caused the dissociation of the alpha-helix structure and broke up of crystalline regions. Thus, the side-chains of amylopectin were hydrated and swollen laterally, further destroy crystalline regions. The starch molecules did not stretch longitudinally leading to a contract to a random coil structure (French, 1984). Previous studies reported, during irradiation, the partial degradation of amylopectin and/or amylose in amorphous regions, causing the decrease of swelling power and the increase of solubility (Radley, 1960; Duarte and Rupnow, 1994).

3.7. In vitro digestibility

The in vitro digestibility of EB irradiated starches were shown in Table 1. Rapidly digestible starch (RDS) was insignificantly changed up to 7 kGy treatment. At 1.0 kGy of irradiation, slowly digestible starch (SDS) was decreased whilst resistant starch (RS) was increased. Furthermore, from 2.7 to 7 kGy, RS was converted to SDS. Theoretically, SDS was proved to control the blood glucose level, reduce the risk of diabetes cardiovascular diseases, colon cancer, and breast cancer (Jenkins et al., 1988). Previous studies determined the changes in digestibility during irradiation (Polesi et al., 2018). Based on the results in this study, there were many factors affected on EB irradiated starch: (i) the depolymerization of starch molecules (Pimpa et al., 2007), (ii) the formation of cross-linkages causing the increase of DRC (Braşoveanu and Nemţanu, 2018), and (iii) the breaking down of large crystals to smaller ones (Brasoveanu et al., 2013). The increase of RDS could be elevated by depolymerization resulting in the formation of smaller molecules that were easily attacked by digestive enzymes. The formation of cross-linkages inhibited enzymatic hydrolysis, thereby reducing the starch digestibility. Furthermore, the crystalline regions positively related to RS fractions (Polesi et al., 2018). Thus, the combination of these factors made it possible to increase or decrease starch digestibility, depending on the factor that predominates.

4. Conclusion

Under EB irradiation, many changes in structural, physico-chemical properties and digestibility of maize starch were characterized. Irradiation dose was an important factor that significant impact on these properties. Base on the treatment dose, many factors affected starch molecules. For each desired condition, the predominant factor was decided appropriate properties of starch. Actually, EB irradiation maize starch showed an advantage in industrial applications such as lower viscosity, higher acidity and solubility comparing to raw starch. Furthermore, the formation of SDS or RS during treatment made it become more beneficial for human health.

Acknowledgment

I would like to express my deepest appreciation to all those who provided me the possibility to complete this study. A special gratitude I give to Ho Chi Minh City University of Technology and Education in stimulating suggestions and encouragement, helped me to finish this study. This work was supported by the Ho Chi Minh City University of Technology and Education.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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