Dual Modification Approach for Tapioca Starch Using Gamma Irradiation and Carboxymethylation

Imtiaz Ali (imtiaz_17003333@utp.edu.my)
Universiti Teknologi PETRONAS

Maqsood Ahmad
Universiti Teknologi PETRONAS

Syahrir Ridha
Universiti Teknologi PETRONAS

Cajetan Chimezie Iferobia
Universiti Teknologi PETRONAS

Najeebullah Lashari
Dawood University of Engineering & Technology

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Abstract

Native starches are modified to enhance their characteristics in terms of thermal stability, cold water solubility, and bacterial susceptibility, which limit their industrial applications. In this work, dual modification of tapioca starch by gamma irradiation followed by carboxymethylation was carried out, and the modified starch characteristics were examined. Four dosages of gamma irradiation (25, 35, 45, and 60 kGy) were used for the first modification stage, followed by carboxymethylation using different parameters. The required modification of starch was characterized by FTIR, SEM, TGA, and XRD. Experimental findings showed that the dual modification enhanced the thermal stability of the starch. In addition, carboxymethylation impacted starch's morphology and reduced its crystallinity. Furthermore, the dual-modified starches exhibited excellent characteristics and could be used in specific applications, including oil and gas, textile, paper, packaging, 3D printing, cosmetics, and pharmaceutical industries.

1. Introduction

Starch is a biodegradable polymer that is increasingly used in many branches of industry because of its respective physicochemical properties. It is naturally occurring in grains, fruits, roots, and tubers of plants and acts as their primary storage material. It is obtained from potatoes, corn, wheat, and rice in separation process. Chemically, starch consists of two polysaccharides amylose and amylopectin. There is a significant structural difference between amylose and amylopectin. Amylose is linear, and amylopectin is a branched polymer. Amylose fraction generally constitutes 20–30% of starch, while amylopectin contributes 70–80%. Several characteristics affects the starch applications, such as particles size, shape, distribution, and crystallinity. Starch is used in non-food products as viscosifier and gelling agent (Adewale, Yancheshmeh, & Lam, 2022; Majzoobi & Farahnaky, 2021; Zarski, Bajer, & Kapuśniak, 2021). However, some industrial utilization of native starches is constrained by their intrinsic characteristics, such as lower thermal stability, cold water solubility, and bacterial susceptibility. This reduces their suitability for wide industrial applications. Hence, there is growing attention to starch modification to acquire appropriate physicochemical and functional properties and improve its appropriateness for industrial applications (Yao Liu, Lu, Hu, Jin, & Miao, 2020).

Starch modification by different mechanisms is attributed to improved physicochemical, functional, and textural characteristics. Various methods of starch modification, such as physical, chemical, enzymatic, or dual modification, have been used so far. Irradiation procedure is one of the developments of nuclear technology that is applied in various fields. It is easily controllable, energy-efficient, leaving no residue, and is an environmentally friendly physical phenomenon (J.-S. Lee, Ee, Chung, & Othman, 2013). The gamma irradiation of starch has recently attracted researchers' interest as a greener, faster, and more sustainable approach to starch modification than conventional chemical methods. Gamma irradiation allows a material modification to perform certain functions. It develops free radicals on starch molecules resulting in changes in starch characteristics. The number of free radicals produced is determined by the moisture content of the starch and the exposure time of storage. An increase in gamma irradiation dosages increases free radicals' intensities on carbohydrates which are responsible for starch molecule
fragmentation and molecular alterations. Irradiation alters the structure of both amorphous and crystalline regions of starch, leading to physical and rheological changes. Numerous researchers highlighted the significance of gamma-irradiated starch for industrial applications (Cieśla & Sartowska, 2016; Dar et al., 2018; Gani, Bashir, Wani, & Masoodi, 2012; Köber, Gonzalez, Gavioli, & Salmoral, 2007; Kurdziel et al., 2022; J.-S. Lee et al., 2013; T. Liu, Ma, Xue, & Shi, 2012; Lu, Donner, Yada, & Liu, 2012; Majeed, Wani, & Hussain, 2017; Salari, Sowti Khiabani, Rezaei Mokarram, Ghanbarzadeh, & Samadi Kafil, 2021; Sunder, Mumbrekar, & Mazumder, 2022; Yoon et al., 2010; Zhu, 2016).

Carboxymethylated starches also present promising characteristics in terms of improvements in starch cold water solubility, thermal stability, and bacterial degradation. It is a feasible approach to improve starch solubility, thermal stability, and shear resistance. Research has been carried out on potato, corn, rice, maize, cocoyam, water yam, and kudzu root starches, showing improvements in the mentioned properties. Numerous researchers have studied the industry's importance and applications of dual modification of starch (Chakka & Zhou, 2020; Das, Jha, & Kumar, 2015; El-Sheikh, 2010; Lamaming et al., 2020; O. S. Lawal, Lechner, & Kulicke, 2008; S. Lee et al., 2010; Milotskyi, Bliard, Tusseau, & Benoit, 2018; Paramakrishnan, Jha, & Kumar, 2016; Sangseethong, Chatakanonda, Wansuksri, & Sriroth, 2015; L.-F. Wang, Pan, Hu, Miao, & Xu, 2010; Y. Wang et al., 2020; Zahib et al., 2021). To the best of the authors' knowledge, there is no literature on the tapioca starch dual modification and its characterization with the current method. This study aims to modify tapioca starch with a dual modification: gamma irradiation and carboxymethylation. The starch thermal resistance, morphology, and crystallinity have been examined. The current modification method is a novel procedure and will be a new input for non-food industries to exploit this ecofriendly technique.

2. Experimental

2.1. Materials

Native tapioca starch was procured from Avantis laboratory supply, Malaysia. The reagent sodium monochloroacetic acid (MCA) was purchased from Sigma Aldrich. Sodium hydroxide (NaOH), acetone methanol, ethanol, and isopropanol were purchased from R&M chemicals. The materials were used without further processing.

2.2. Starch gamma irradiation

Native tapioca starch (TS) was dried naturally for ten days in the sunlight before being subjected to gamma radiation to remove the moisture content (<10%). This was followed by artificial drying for 48 hours at 122 °F. The starch was treated by gamma-irradiator (NORDION Cobalt 60 Irradiator Model: JS10000) at different doses of 25, 35, 45, and 60 kGy (100 rad = 1Gy). The dosage rate was maintained at 1kGy per 40 minutes. The dosimetry was performed using Ceric Cerous manufactured and calibrated by SSDL, Nuclear Malaysia.

2.3. Irradiated Starch Carboxymethylation
For carboxymethylation, a standard procedure was followed with some modifications in reaction conditions. Briefly, a 500 mL flask was filled with deionized water, and various amounts of sodium hydroxide (6.48–8.08 g) were added. The mixture was stirred at 300 rpm until the NaOH was completely dissolved. Isopropanol was added to the mixture, and the temperature was set up to the required temperature. Taking the moisture content of tapioca starch (8.9%), the reaction mixture water content was adjusted between 10 to 25 mL. Starch (9.4 g) was added to the mixture and stirred at 300 rpm while nitrogen gas was pumped through the mixture. MCA was added to the mixture after 1 hour of stirring, and the reaction time was adjusted to 30, 60, 120, 180, and 240 minutes. The starch mixture was filtered at the end of the process, suspended in ethanol, then dispersed again in 85% ethanol, and rinsed thrice until the silver nitrate test for chloride in the filtrate turned negative. Finally, the resultant mixture was suspended in ethanol, washed multiple times, and dried for 48 hours in a 122 °F oven.

2.4. Factors affecting carboxymethylation reaction

The primary reaction parameters that are known to influence the carboxymethylation process were investigated. Twenty-five (25) experimental runs were performed based on considering five parameters. The reaction parameters were considered as reaction time (30, 60, 120, 180 and 240 minutes), reaction temperature (86, 104, 122, 140 and 158 °F), water to solvent ratio (0.04, 0.08, 0.12, 0.16 and 0.20), nNaOH/nAGU (0.40, 0.81, 1.21, 1.62 and 2.02) and nMCA/nAGU (0.36, 0.72, 1.08, 1.44 and 1.80).

2.5. Degree of Substitution (DS)

The degree of substitution (DS) is the number of carboxymethyl groups generated in each anhydroglucose unit (AGU) of a starch. Since every AGU has four hydroxyl groups and the value of DS lies in the 0–3 range. The degree of substitution was measured using the direct titration method, the most popular chemical assessment technique that can give an accurate assessment of the DS. For DS determination, 5 g of carboxymethyl starch was dispersed in 150 mL acetone, then 15 mL (5M HCl) was mixed with the suspension and agitated for 30 min. The Na-CMS was turned into H-CMS (protonated CMS) in this step. The product was rinsed with 80% methanol five times till the pH was neutral. The mixture was filtered, immersed in acetone, and stirred for 15 min. Finally, the dispersion was filtered and dried for 24 hrs at 122 °F. 2 g protonated CMS was dissolved in 1% (w/v) NaCl solution by using phenolphthalein as an indicator. The volume of sodium hydroxide was recorded, and the value of the degree of substitution (DS) was evaluated using the following equations (J. Liu, Chen, Dong, Ming, & Zhao, 2012).

\[
DS = \frac{M_o \times n_{NaOH}}{m_c - n_{NaOH} \times MR} \# \quad (1)
\]

\[
m_c = m_p - \left[ \frac{m_p \times F}{100} \right] \quad \# \quad (2)
\]

Where \(DS\) is the degree of substitution, \(Mo\) represents the molar mass of the AGU (g/mol), \(MR\) is the molar mass of carboxymethyl residue (g/mol), \(nNaOH\) is the amount of NaOH used (mol), \(mp\) is the weight of the polymer taken (g), \(mc\) is the corrected weight of the polymer (g), and \(F\) is the percentage of moisture.
2.6. Characterization

Four characterization techniques were used to confirm the starch modification using the dual modification method.

FTIR spectroscopy was used to determine and validate the conformational changes of starch compared to its native form. The FTIR instrument (Perkin Elmer, Model: FTIR Frontier) was used to record starch samples' FTIR spectra before and after dual modification. Samples analyses were carried out by scanning a wavelength range of 4000 to 400 cm$^{-1}$ with 16 scans (resolution: 4) for obtaining absorbance percentage. The starch sample (5%) was adequately mixed with potassium bromide (KBr) through a mortar and pestle. The resultant mixture was evacuated and compressed with a force of ~ 70 kN (7 Ton) for ~ 3 min. The resulting pellets were then used to measure the spectra in the transmission mode. The transmittance spectra were transformed into absorbance spectra after baseline adjustment. Infrared spectroscopy identified chemical functional group vibrations in the studied samples.

Morphological characteristics of the native and CMITS samples were studied using a scanning electron microscope (Model: ZEISS - EVO LS 15, Carl Zeiss, Germany). The samples were dried before testing, placed on a metal stub, and coated with gold to make the samples conductive. Micrographs were captured at an accelerating voltage (20 kV), high vacuum, and various magnification levels.

The thermogravimetric analysis of native and CMITS samples was accomplished with a Perkin Elmer STA 6000 under a nitrogen atmosphere. 5 mg sample was placed in the container and heated at a rate of 10 °C/min under nitrogen (N$_2$) at a rate of 20 mL/min from 30 °C to 800 °C. The obtained data in terms of TGA and DTG curve was plotted, where TGA is the mass loss ratio, and DTG represents the mass loss rate.

The XRD apparatus (Model PAnalytical Xpert Powder) was used to record the X-ray diffraction patterns of native and CMITS samples. Scanning was performed from 5–80° (2θ) at operating conditions (40 kV and 40 mA). The XRD analysis was carried out using a built-in tool (X’Pert HighScore Plus).

3. Results And Discussion

3.1. Carboxymethyl Irradiated Starch Characterization

The gamma irradiation dosages caused various changes in native starch properties. The water absorption capability was increased, whereas swelling capacity decreased after the gamma irradiation in a dose-dependent approach. This was attributed to the fact that higher gamma irradiation dosage caused starch degradation. Before carboxymethylation, the samples' moisture content was measured, which was found below 10% for each studied sample. The starch samples before and after irradiation treatment are given in Fig. 1. The color of the native starch was changed to yellowish as the irradiation dosage was increased.
3.2. Influence of Reaction Parameters

Generally, the nature of chemicals chosen to treat starches is limited by their inadequate solubility. For starch modification, monochloroacetic acid (MCA) was used in the presence of sodium hydroxide (NaOH). NaOH dissolved starches efficiently and delivered appropriate reaction conditions for functionalization, allowing for a consistent etherification process. The influence of various factors, including the molar ratio of NaOH, molar ratio of MCA, reaction temperature, reaction time, and solvent type, on the degree of substitution (DS) was examined.

3.2.1 Effect of NaOH Concentration on DS

The effect of several molar ratios of NaOH to starch (AGU) on the DS value is presented in Fig. 2. The influence of sodium hydroxide on DS was evaluated by changing the sodium hydroxide concentration. It was found that with the addition of NaOH to the reaction, the product increased the DS value until the DS value reached a maximum of 0.64 at a molar ratio \( \frac{n_{NaOH}}{n_{AGU}} \) of 1.62. However, further increasing amounts of NaOH resulted in a reduction in the product's DS.

It is a general approach to use an alkaline solution to enhance the nucleophilicity of hydroxyl groups in starch, which facilitates carboxymethylation reaction. The optimum molar concentration of NaOH (1.62) facilitated the starch swelling. It increased the number of starch-O-Na, leading to a considerably higher risk of carboxymethylation in the starch molecules containing the OH group. Nevertheless, at a higher NaOH dosage than \( \frac{n_{NaOH}}{n_{AGU}} = 1.62 \), a side reaction of monochloroacetic acid with sodium hydroxide also occurred, resulting in the decline of DS value. This result can be explained by looking at the carboxymethylation procedure, which simultaneously involves two duelling processes. In the synthesis of CMITS, the starch hydroxyl first reacts with monochloroacetic acid in the presence of sodium hydroxide, according to Williamson's ether synthesis (Yanli, Wenyuan, & Xia, 2009).

\[
\text{NaOH + Cl-CH}_2\text{COONa} \rightarrow \text{HO-CH}_2\text{COONa} + \text{NaCl}
\]

Then, in the subsequent process, sodium hydroxide reacts with monochloroacetic acid to form sodium glycolate.

According to the current findings, an optimum NaOH dosage is required for the carboxymethylation process, and the optimum NaOH/AGU molar ratio was 1.62 when the other parameters were kept
constant. This ratio is more appropriate for the reaction, which reduces undesirable secondary reactions in the current research.

Compared with the non-irradiated starch, the irradiated starch showed better performance in terms of the degree of substitution. The increase in the irradiation dosage also improved the starch carboxymethylation process. The higher DS value was obtained when the 45 kGy irradiated starch was carboxymethylated. After this dosage, the starch granules were agglomerated, and the carboxymethylation reaction was ineffective. In addition, the solubility of the starch after 45 kGy was reduced, which reduced the significant conversion of starch and hindered the reaction. The findings showed that the sodium hydroxide concentration improved the DS value of the 60 kGy sample almost linearly compared with other dosages. It may be due to the increase in swelling due to NaOH concentration that facilitated the starch particles swelling and developed additional starch-O-Na.

### 3.2.2 Effect of Etherifying Agent on DS

Carboxymethyl groups are attached to the starch using monochloroacetic acid (MCA) as an etherifying agent at various concentrations. Similar to NaOH concentration, the ratio of $n_{\text{MCA}}/n_{\text{AGU}}$ also affected the value of the degree of substitution. Initially, the increased MCA also increased the DS. The maximum DS value was observed as 0.56 with the $n_{\text{MCA}}/n_{\text{AGU}}$ value of 1.08 at an optimum molar ratio of $n_{\text{NaOH}}/n_{\text{AGU}}$. With a further increase in the ratio, the DS value declined due to MCA and NaOH's side reaction, leading to sodium glycolate formation. The irradiated starch showed better performance when compared with the non-irradiated tapioca starch. The results indicated that the DS is improved with the increase in the irradiation dosage. It further shows that the irradiated starch had a higher DS value suggests that irradiation appears to activate the starch to react with monochloroacetic acid. The irradiation dosage increased the DS value until 45 kGy, but a further increase in irradiation caused a reduction in the DS. The reason is the destruction of starch granules due to the higher gamma irradiation, which reduced its efficiency in reacting with the reagents.

### 3.2.3 Effect of Water Content on DS

Carboxymethylation was carried out using isopropanol as a medium, as it is an appropriate organic medium in carboxymethylation reactions. The amount of water to isopropanol ratio influenced the degree of substitution. It played various roles, including facilitating starch swelling and dissociation, diffusion, and adsorption of the etherifying agent. The water content was changed by keeping the other conditions constant as temperature 122 °F, reaction time 180 minutes, $n_{\text{MCA}}/n_{\text{AGU}}$ 1.08, and $n_{\text{NaOH}}/n_{\text{AGU}}$ 1.62. It initially improved the DS, but with a further increase, the DS value declined. The findings showed the optimum DS value was obtained when the water to isopropanol ratio was 0.16. It has been noticed that after increasing the ratio from 0.16, no change has been observed.
Out of all the studied starches, the degree of substitution of the 45 kGy starch showed higher values, whereas the 60 kGy starch showed the minimum DS value. The reason could be the higher irradiation dosage which altered the starch granular properties and could not resulted the better results. On the other hand, the higher water to solvent ratio caused starch gelation, which further decreased interaction between the etherifying agent (MCA) and the starch granules. Moreover, the starch morphology was altered, making the ultimate recovery challenging. The non-irradiated starch showed better results than the 60 kGy sample. It is because the starch granules were destructed during the higher dosage of the irradiation, which was difficult to dissolve in the water under the mentioned conditions.

3.2.4 Effect of Reaction Duration on DS

Reaction time is another important parameter that allows the etherifying agents to react with the starch. In the current case, starch swelling and, subsequently, the reactants' homogeneity were enhanced by the longer reaction time (Fig. 5). The DS was increased with the increase in the duration of the reaction. After reaching an optimum value, the DS remains unchanged. It has been noticed that the optimum reaction duration was 180 minutes. After this time, no variation in the DS value was noticed, showing that the maximum reagents have been reacted with the starch and no more reaction is possible with the increase in the reaction duration. It can be predicted that the accessibility of etherifying agents has a maximum DS value regardless of how long it takes. It is due to the higher swelling of the starch.

3.2.5 Effect of Temperature on DS

Temperature is known to enhance the percentage of molecules with greater energy than the activation energy, and the DS improves. The temperature was revealed to have an impact on both the swelling of starch granules and the diffusion of the reactants in the reaction. As the temperature rises, the percentage of molecules whose energy exceeds the activation energy increases, increasing the reaction rate and hence the DS. The optimum temperature at which the maximum DS value was obtained was 122 °F for 45 kGy sample (Fig. 6), while the other starches showed better DS values at 60 and 158 °F. Afterward, no significant changes were found with a further increase in reaction temperature. The DS of the non-irradiated starch increased as the temperature rose. Due to the gelatinization temperature of the starch, the stirring was difficult, which caused agglomeration resulting in difficulties in product recovery. The gelatinization temperature of the native tapioca starch was reported to be between 153–158 °F (Ratnayake & Jackson, 2007, 2008; Zhang, Tong, Zhu, & Ren, 2013), which shifted to a higher value due to the irradiation dosage.

In all of the above experiments, various doses of irradiation starch were carboxymethylated under varied reaction conditions. It was observed that DS was increased when irradiation dosage was increased in the carboxymethylation process. It is due to the activation of starch granules after irradiation to react with the reagents. As carboxyl groups are formed due to gamma-irradiation and the carboxyl group content increased with the increase in irradiation dose (Chung & Liu, 2009; Verma et al., 2018). In addition, the
starch acidity was found to increase when the radiation dose was raised. The rise in carboxyl content of irradiated starch might be attributed to free radical action breaking down the starch molecules, probably generating carboxyl groups to develop. Slower irradiation dosage rates may result in lower carboxyl content than a high dosage rate. Additionally, irradiation of native starch may produce carboxylic acids, which increase the carboxyl content and lower the pH value of irradiated starch samples. These carboxyl groups influence the DS of samples. However, the higher irradiation dosage above 45 kGy showed adverse effects on the DS of the starch. It is due to the fact that the γ-irradiation clings the polysaccharide chains, which causes the reduction in the degree of polymerization.

The amylose content of starch is an essential indicator of starch quality, which is influenced by γ-radiation. It seems that a low dosage of radiation may result in the degradation of amylopectin (which is significantly larger than amylose). At higher doses, additional amylose and amylopectin degrade by radiation resulting in chain fragments. In addition, the lower dosage had a minor effect on the apparent amylose starch content.

The experimental results indicated that the effectiveness of carboxymethylation depends on the swelling and carboxymethylation agent penetrating the starch granular structure. Carboxymethylation of starch also influences the structural properties of the starch. Chemical modification reactions only occur in the amorphous regions of starch granules because it is challenging to introduce chemicals into the crystal region. However, Y-rays may degrade any part of the starch granule (both the amorphous and crystalline areas). According to the literature (Wu & Song, 2006), after γ-irradiation, starch loses most of its crystallinity, increasing the amount of reagents diffused into the starch granule structure. As a result, the DS value increases and ultimately the starch becomes water soluble when the DS is greater than 0.1 (Roberts, 1967).

3.3. Characterization

The following characterization techniques have been used to confirm the required modification by using the dual modification method.

3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of native and irradiated carboxymethyl tapioca starch (at different DS values) are shown in Fig. 7. The intensity of native starch features has been influenced by carboxymethylation, that has induced the formation of new bands. In the IR spectrum of native starch (Fig. 7 (a)), the extremely broad band between 3000 to 3750 cm⁻¹ was attributed to the OH stretching vibrations. The measurements indicated the desired starch modification, which was carried out. The FTIR spectrum of modified starch (DS 0.56) is shown in Fig. 7 (b). In Fig. 7 (a), the hydrogen-bonded hydrogels formed by the polysaccharide chains account for the broad band at 3000–3750 cm⁻¹. This band intensity was reduced after carboxymethylation. However, the DS value also affected the intensity of this band. Similarly, a band at 2933 cm⁻¹ was attributed to CH₂ symmetrical stretching vibrations. This peak has not
been affected significantly after carboxymethylation. Likewise, the carboxymethyl starch spectrum showed strong COO- vibration absorption peaks at around 1435, 1433, and 1429 cm\(^{-1}\) in the double bond range (Fig. 7 (b, c, and d)). Similar trends have also been reported by J. Liu et al. (2012) for potato starch.

### 3.3.2 Morphological and Surface Behavior Analysis

Figure 8 shows the micrographs of native and carboxymethyl starches. The morphologies of native and modified starch indicated that native tapioca starch granules had a spherical, ellipse, and polygonal morphology and a normal granule size distribution. Furthermore, their surfaces were smooth and circular, with a truncated end on one side and diameters varying from 2 to 45 µm. The size variation in native starches occasionally occurs due to starch extraction and drying procedures. The native starch surface showed no fissures or pores on it. Whereas, in the modified starch, the granular structure of starch was entirely changed, as demonstrated in Fig. 8 (b, c, and d). The \(\gamma\) Irradiation produced free radicals in starch that interacted with water and starch molecules to affect the molecular and granular structures of the starch. When compared to native starch, irradiation and carboxymethylation treatment induced various structural variations in starch granules. The edges of the CMITS 0.64 and 0.66 granules lost considerable clarity, and the surfaces were found coarse-textured. Furthermore, the sides of these particles were porous, with numerous cavities. Additionally, changes in isopropanol aqueous solution environments moderately affected the starch granular structure.

Similar morphological changes have also been observed in the granule morphology of other carboxymethyl starches reported in the literature (Olayide S Lawal, Lechner, Hartmann, & Kulicke, 2007; Xu et al., 2017). Researchers reported that starches exposed to higher alkaline conditions during carboxymethylation are primarily responsible for this change. The current findings also indicated that the crystallinity of starch was affected due to irradiation as well as the alkaline environment, which allowed the etherifying agents for the carboxymethylation process to have further accessibility to the starch molecules. The reaction has caused morphological changes and altered the granular shape of the starch as the amorphous region of the material increased, which was also observed in XRD results.

### 3.3.3. Thermogravimetric Analysis

The TGA and DTG curves for NTS and its modified forms are shown in Fig. 9. Figure 9 (a) shows the thermal stability of NTS sample where the first weight loss (~ 6%) begins around 30 °C to 70 °C and may be due to water molecules. The subsequent weight loss ends around 142 °C, corresponding to a 5% loss. It is due to the moisture contents and the water loss from starch due to the dehydration reaction between the hydroxyl groups in NTS. This suggests that the starch will be more stable when the minimum hydroxyl groups are left on the starch. It implies that a starch derivative would produce a stable molecule with minimum -OH group content (X. Li et al., 2010; Rudnik, Matuschek, Milanov, & Kettrup, 2006). The last and significant degradation lies in the range of 250–304 °C. The DTG curves too exhibit significant
weight loss at ~ 300 °C for native starch. The native starch degraded at a lower temperature than the CMITS, and with the increase in temperature, starch was completely degraded.

Figure 9 (b) shows the TGA and DTG curves for CMITS (DS 0.64). It illustrates two degradation stages, where the initial weight loss lies in the range of 30 °C to 130 °C. It is due to the residual methanol and water that are leftover post washing (Castaño, Bouza, Rodríguez-Llamazares, Carrasco, & Vinicius, 2012). The next weight loss lies between 250 °C to 365 °C related to the starch structure breakdown. It is evident that the maximal weight loss for CMITS starch has switched to a higher temperature (above 300 °C), indicating that the thermal stability after carboxymethylation has improved.

When the degree of substitution was increased to 0.66, the thermal stability of the sample was also improved, as shown in Fig. 9 (c). The same two-stage degradation is observed in the sample. In the first stage, the first weight loss was observed at around 30 °C to 135 °C and is due to water/methanol, and the next weight loss was between 250 °C to 385 °C corresponding to the degradation of the starch structure. Similar to the previous sample, the weight loss has moved to a higher temperature, which demonstrates the increase in the thermal stability after carboxymethylation. An increased thermal stability of the starch was possibly a result of higher molecular weight and more stable microstructures owing to added groups, which require more energy to break the molecular starch chains (X. Liu et al., 2013; Ying Liu, Yang, Ma, & Zhang, 2019; Ricky, Mpelwa, Wang, Hamad, & Xu, 2022). The findings also indicate that CMITS decomposes earlier than native starch, although at a slower rate.

### 3.3.4 XRD Analysis

The crystalline structure of native and modified starch specimens in terms of diffractograms are given in Fig. 10. A characteristic A-type crystallinity pattern was seen in the diffractogram of NTS, which is consistent with the literature (Dome, Podgorbunskikh, Bychkov, & Lomovsky, 2020; Katsumi et al., 2015; Othman, Hassan, & Hashim, 2015). Starch crystallinity was substantially reduced after carboxymethylation because of the alkaline environment and the presence of water that allowed the starch granules to rupture. For the native sample. The pattern has strong reflections at 14.96, 17.14, 17.93, and 23.08° of a diffraction angle (2θ). These peaks are particularly indicative of crystalline regions in the starch (B.-z. Li, Wang, Li, Adhikari, & Mao, 2012; Zhong, Kong, Chen, Grady, & Qiu, 2021). These visible reflections disappeared after chemical modification Fig. 10, which revealed that the crystalline features in the starch molecules had been abolished. Similar patterns have also been reported previously for other starch carboxymethylation (Kittipongpatana, Chaitep, Charumanee, & Kittipongpatana, 2006; Yanli et al., 2009). Since the crystallinity of native starch was affected in strongly alkaline conditions, etherifying agents were able to obtain better access to starch molecules for the carboxymethylation reaction.

**Conclusion**

The thermal stability, morphology, and crystallinity of the native tapioca starch have been improved with the dual modification process. The results showed that 45 kGy is the optimal dosage rate at which the
maximum degree of substitution has been achieved. Furthermore, the modified starch's thermal stability was found to be enhanced, and the higher degree of substitution resulted in higher thermal stability, which confirms the addition of carboxyl groups into the starch. The FTIR results confirmed the required modification by showing the carboxyl groups in the modified samples. The degree of substitution slightly affected the peaks. The morphology of the modified starch was changed, and the smooth edges of the starch granules were changed to sharp edges. Similarly, the crystallinity of the modified samples also vanished after modification.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MCA</td>
<td>Monochloracetic acid</td>
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<tr>
<td>kGy</td>
<td>Kilogray</td>
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<tr>
<td>DS</td>
<td>Degree of substitution</td>
</tr>
<tr>
<td>CMITS</td>
<td>Carboxymethyl irradiated tapioca starch</td>
</tr>
<tr>
<td>AGU</td>
<td>Anhydroglucose unit</td>
</tr>
<tr>
<td>CMS</td>
<td>Carboxymethyl starch</td>
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<tr>
<td>NTS</td>
<td>Native tapioca starch</td>
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References


Figures

(a) Native starch, starch irradiated at (b) 25 kGy, (c) 35 kGy, (d) 45 kGy and (e) 60 kGy
Figure 2

Effect of $n_{NaOH}/n_{AGU}$ on DS
Figure 3

Effect of $n_{MCA}/n_{AGU}$ on DS
Figure 4

Effect of Water/Isopropanol Ratio on DS
Figure 5

Effect of reaction duration on DS
Figure 6

Effect of temperature on DS
Figure 7

FTIR Spectra of native (a) and modified starches (b, c, and d)

Figure 8

SEM Micrographs of (a) native and (b) CMITS 0.56, (c) CMITS 0.64 and (d) CMITS 0.66 starch samples
Figure 9

TGA and DTG curve of (a) native tapioca starch (NTS), (b) CMITS 0.64 and (c) CMITS 0.66
Figure 10

XRD curves of native and modified CMITS samples