Starch recovery from turmeric powder (*Curcuma longa*) after ethanol curcumin extraction in comparison to the conventional method

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ABSTRACT

Recovery starch from organic waste significantly contributes to sustainable agricultural production. This study aimed to recover starch from the waste generated from the curcumin extraction by using ethanol. The physicochemical properties of the isolated starch such as microscopic morphology, Fourier transform infrared spectroscopy, X-ray diffraction, total starch, iodine binding capacity of starch, curcumin content determined by high-performance liquid chromatography were compared to that of starch obtained from the conventional method of extraction from the fresh rhizome. The results showed that the starch obtained from the fresh rhizome had a higher yield compared to that of starch isolated from the turmeric powder after extracting curcumin (21.3% vs. 8.5%). The total starch analysis indicated that the former starch had a higher purity (98% vs. 77%, dw). The SEM imaging showed that both starches had irregular shapes with a thick flat and smooth surface. Although the starch isolated from the turmeric powder showed the dedicated properties of starch, the peak intensity and crystalline structure were remarkably decreased, via FTIR and X-ray diffraction analyses, respectively. The pasting analysis showed a clear change in starch obtained from the turmeric powder after ethanol extracting curcumin since a low peak viscosity was recorded. The HPLC curcumin quantification showed that both starches had a very low residue of curcumin (18.4 mg/100 g and 66.5 mg/100 g, dw). The process of starch recovery after curcumin extraction from turmeric would be further improved to prevent the changes in physicochemical properties and for better yield.

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1. Introduction

Tumeric (*Curcuma longa*) is commonly distributed in tropical and sub-tropical regions. In Vietnam, turmeric is widely cultivated in northern and highland areas. One of the most significant components of the rhizome is the curcuminoids that have been intensively investigated. Curcuminoids from turmeric have been reported as a natural food colorant, preservative, and *in vitro* anticancer (Yu & Huang, 2010), antioxidants and inflammatory agents (Anand et al., 2008).

Starch is one of the major components in the turmeric rhizome. Starch accounts for 47% of the dried weight of the rhizomes (Leonel et al., 2003). In another study, the turmeric rhizome contained approximately 67% starch (dw) (Kuttigounder et al., 2011). Regardless of its high starch content, the application has been seldom extended in the food and pharmaceutical industries. According to Santana et al. (2017), turmeric starch is essential as an alternative starch source for the food industry. Although several researchers

have been addressed on isolation and characterization of the physicochemical properties of starch from fresh turmeric rhizome (Jyothi et al., 2003; Kuttigounder et al., 2011; Sajitha & Sasikumar, 2015), the recovery starch after curcuminoids extraction seem very limited. It has been reported that turmeric starch was successfully recovered from supercritical fluid and would be essential use for dietary starch (Santana et al., 2017).

Ethanol extraction of curcuminoids has been utilized as a green method of solvent extraction from the turmeric powder (Osorio-Tobón et al., 2016; Lateh et al., 2019; Patil et al., 2019). However, starch recovery from the residue of ethanol curcumin extraction has not been investigated yet. The present study aimed to isolate starch from turmeric powder after curcumin extraction by using ethanol. The physicochemical properties of the isolated starch were then analyzed and compared to those of the starch obtained from the conventional method of extraction from the fresh turmeric by precipitation to evaluate its potential application in the industry.

2. Materials and Methods

Materials: the mature (at least one-year-old) yellow turmeric rhizomes (*Curcuma longa*) were procured from Ea Bhok, Cu Kuin district, Dak Lak province, Viet Nam.

Chemicals: Curcumin (Himedia, India), acetonitrile, orthophosphoric acid, ethanol (HPLC grade, Merck) were obtained from the local supplier. Hydrochloric acid (37%), iodine, sodium hydroxide pellet, dimethyl sulfoxide (analytical graded) were purchased from a local supplier.

2.1. Isolation of starches

2.1.1. Isolation turmeric starch from the fresh rhizome

The procedure of isolating starch from fresh turmeric rhizome followed the procedure developed by Nakkala et al. (2020) with modifications. The rhizomes were cleaned and sliced into slices (around 2 mm thick) using a domestic slicer. The turmeric slices were then immediately soaked and ground in sodium metabisulfite 0.02% (w/v) (the ratio of turmeric and sodium metabisulfite was 1:4). After grinding, the turmeric was soaked in the sodium metabisulfite for further 12 hours. The finely ground sample was then filtered through a cheesecloth to collect the solution containing starch. The retained portion was re-slurried with 10 L water and filtered for the second time. The solution collected from the second filtration was pooled with the first solution. The starch was precipitated overnight without disruption. The clear white-yellowish layer of turmeric starch was decanted and dried in a heat pump dryer at a temperature of 35°C until reached the moisture content of approximately 10%. The dried starch was passed through a 300 µM sieve and the starch powder was stored in a sealed aluminum bag at room temperature for further analysis.

2.1.2. Isolation of starch from turmeric powder after ethanol curcumin extraction

To mimic the starch recovery from waste generated from the curcumin extraction process, the turmeric rhizomes were initially curcumin extracted. The slices of turmeric rhizome (similarly prepared as aforementioned) were dried in a heat pump dryer at 35°C until reached the moisture content around 10%. The dried turmeric slices were then pulverized into powder by using a domestic grinder. To avoid overheating, the grinder was intermittently stopped for 30 sec for every 30 sec-grinding process. The ground sample was passed through a 300 µM sieve. The fine turmeric powder was extracted with absolute ethanol with the ratio of 1:100 at 50°C for 8 h to mimic the curcumin extraction process. After the extraction process, the sample was filtered through a filter paper and the residue was collected for starch extraction. The sample was re-slurried into 0.02% (w/v) sodium metabisulfite with the ratio of 1:4 and soaked overnight. The slurry was filtered through a cheesecloth. The retained samples were then mixed with 0.02% (w/v) sodium metabisulfite and refiltered. The starch solution was combined and the starch was precipitated, dried at 35°C, passed through a sieve again as previous procedure. The starch powder was put in an aluminum bag, sealed, and stored at room temperature for further analysis.

2.2. Determine total starch

The total starch was estimated based on the acid hydrolysis method (Kent-Jones & Amos, 1960) with modifications. Isolated starches of 2.5 g were hydrolyzed in 220 mL of 3.36% (v/v) hy-

drochloric acid solution in a flask. The starch mixture was heated to 90°C for 2.5 h and then cooled before being neutralized with NaOH 5 N. The volume of the acid hydrolysate was adjusted to the final volume of 250 mL using deionized water. The sugar content in the solution was determined by the colorimetric method using DNS reagent as suggested by (Başkan et al., 2016).

2.3. Iodine binding capacity

The iodine binding capacity of isolated starches was evaluated following the method described by Peng & Perlin (1987). Starch (50 mg) was dispersed in 5 mL dimethyl sulfoxide (DMSO) by heating in a 100 mL volumetric flask to obtain a clear slurry. Then the volume was adjusted to 100 mL. Two milliliters of the diluted starch slurry were transferred into a second volumetric flask (50 mL) in which 1 mL of NaCl 1 M, 40 mL of water, and 1 mL of iodine solution (containing a mixture of 2 mg I_2 and 20 mg of KI). The final volume was brought to 50 mL. The color was developed in 30 min and then the absorbance at 600 nm was measured by using a spectrophotometer and a 1 cm cuvette. The blue value (BV) was calculated based on the recommended equation: BV = (4 x A_{600})/C where A_{600} is the absorbance at 600 nm and C is the concentration of the starch (mg/L) in the solution.

2.4. Scanning electron microscopy (SEM)

The isolated starches were spread on a metal stub attached with a double-sided adhesive carbon tape. The samples were coated with a thin layer of Pt. The imaging was acquired by using FE-SEM S4800 (Hitachi, Japan). The coated samples were imaged in a scanning electron microscope (SEM), Hitachi S-3400 (Tokyo, Japan) at an accelerating voltage of 10 kV and a working distance of 8.0 mm.

2.5. Pasting properties

The pasting properties of the isolated starchers were investigated using Brookfield Engineering Labs (DV2T) system followed the procedure of Rapid Visco Analyzer (RVA) with modifications. The starch solution (7%, w/v) was prepared in water, mixed and then subjected to the system for measurement with the protocol as follows: the spindle speed was kept at 160 rpm. The length of the analysis was 12.5 min. The temperature was kept at 55°C from the first 2 min, followed by an increase from 55 to 90°C in the next 3 min. From 5 - 9 min, the temperatures remained at 90°C before cooling down to 55°C at 12.5 min. The pasting profile was acquired and the viscosity was presented in centipoise (cP).

2.6. Fourier transform infrared analysis

The short-range order structure of starches was examined based on the technique of Fourier transform infrared (PerkinElmer MIR/NIR Frontier). The sample was prepared with KBr and the FTIR spectra were recorded in the range of 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1} per scan.

2.7. X-ray diffraction analysis

The crystalline pattern of isolated starches was analyzed by X-ray diffraction (Bruker D2 Phaser, Germany). The instrument was equipped with a copper X-ray generator working in conditions of 40 kV and 80 mA. 2θ range of 4–60° was used to acquire for X-ray diffractograms with a step size 2θ of 2.0°/min.

2.8. Curcumin analysis by high-performance liquid chromatography

Curcumin in turmeric powder and starches were quantified by using high-performance liquid chromatography (HPLC) following the suggested procedure from Moorthi et al. (2013) with modifications. Sample (50 mg) was extracted in 20 mL ethanol 60% (v/v) in a 50 mL centrifuge tube. The tube was then vortexed for 30 sec for every 15 min in total 2 h. The sample was then centrifuged (4000 rpm, in 10 min) to obtain a clear supernatant. The clear supernatant was filtered through a PTFE (0.45 µM) membrane and transferred into an amber HPLC vial for analysis. HPLC system (Shimadzu, Japan) was equipped with LC20-AD pump, CBM-20Alite controller and PDA detector. C18 column (Inertsil-ODS 3, 250 x 4.6 mm, 5 µM) was used as stationary phase. The mobile phase was a mixture of orthophosphoric acid (0.1%) and acetonitrile (45:55, v/v) with the isocratic flow at 1 mL/min. The curcumin was detected at 427 nm. Curcumin standard (Himedia, India), was used to generate the calibration curve for quantification of curcumin in the samples.

3. Results and Discussion

3.1. Yield and total starch of the isolated starches

The yield of isolated starches were presented in Figure 1. The yield of starch isolated from the fresh turmeric rhizomes was around 21.3% (dw). This yield was significantly higher ($P < 0.01$) compared to that of starch isolated from the turmeric powder after extracting curcumin by ethanol (21.3% vs. 8.5%, Figure 1A). The low recovery of starch from the turmeric powder after ethanol extraction was possibly due to during the drying process of turmeric powder preparation, the starch was entrapped within the cellulosic pockets. Moreschi et al. (2006) observed the turmeric rhizome under the SEM reveal that starches allocate within the cellulosic pocket. Thus, in this study, although the turmeric powder was ground into approximately 300 µM particles, the starch seems still to be kept in the cell wall and limited the escape during starch extraction. The yield obtained from the fresh rhizomes is comparative to that reported in a study by Nakkala et al. (2020). However, in general, the starch yield was lower than that recorded in other studies in which the yield ranged from 40-60% (Leonel et al., 2003; Kuttigounder et al., 2011; Sajitha & Sasikumar, 2015).

The results of total starches are presented in Figure 1B. The starch isolated from the fresh rhizome had a higher total starch content compared to that collected from the turmeric powder after extracting curcumin with ethanol (98.3% vs. 77.2%, dw). The finding suggests that the starch isolated had a better purity compared to that of starch obtained from the turmeric powder. This result agreed with the previous report since the starch has been recovered from the fresh rhizome has a high purity (Alcázar-Alay & Meireles, 2015). The starch isolated from the fresh turmeric rhizome, however, had a higher total starch content reported earlier (ranges from 77-87%) (Leonel et al., 2003; Braga et al., 2006). The isolation of starch from the turmeric powder after extracting curcumin was in an attempt to recover starch from organic waste generated from the industry of curcumin extraction. The low yield and total starch of this starch suggest an improved method for starch recovery.

3.2. Iodine binding capacity of isolated starches

The iodine binding capacity of starch mostly involves the complex of amylose and iodine. The iodine capacity may act as an index for the apparent amylose content of the starch. For the native starch, particularly after isolation, the amylose leaching would be limited and the starch has a high blue value. The blue value of the starch isolated from the fresh rhizome was significantly ($P < 0.01$) higher than in the starch isolated from the turmeric extraction residue (0.589 vs. 0.123) (Table 1). The blue value of starch obtained from fresh turmeric was slightly higher than reported in the literature (blue value of 0.427 in Pham & Vo (2017)). A low blue value was observed in the starch isolated from the turmeric powder that would be involved in the process of extraction. In this study, the turmeric powder was extracted with ethanol for 8 h at 50°C. At these extraction conditions, the starch in the powder appears to be annealed resulting in changes of physicochemical properties, as an example, reduced in the blue value. Lan et al. (2008) reported that the annealing process of starch occurred when starch was treated under the gelatinization temperature of the starch. The annealed starch reduces in iodine binding capacity due to rearrangement at the molecular level, particularly the amylose molecule.

Table 1. The blue value of starches isolated from the fresh rhizome and turmeric powder

Starch	Blue Value
Isolated from the fresh turmeric rhizome	0.589 ± 0.03^{a}
Isolated from the turmeric powder after curcumin extraction	0.123 ± 0.01^{b}

Values are expressed as mean ± SD of triplicates.
Values followed by different lowercase letters in superscripts were significantly different at $P < 0.01$.

3.3. Scanning electron microscopic imaging (SEM)

The morphology of the starch was observed under the SEM and the images are presented in Figure 2. The turmeric starch had an irregular, nearly oval and flat shape. The starches had a smooth surface and were around 5 µM thick. The starches granules can be classified into the small

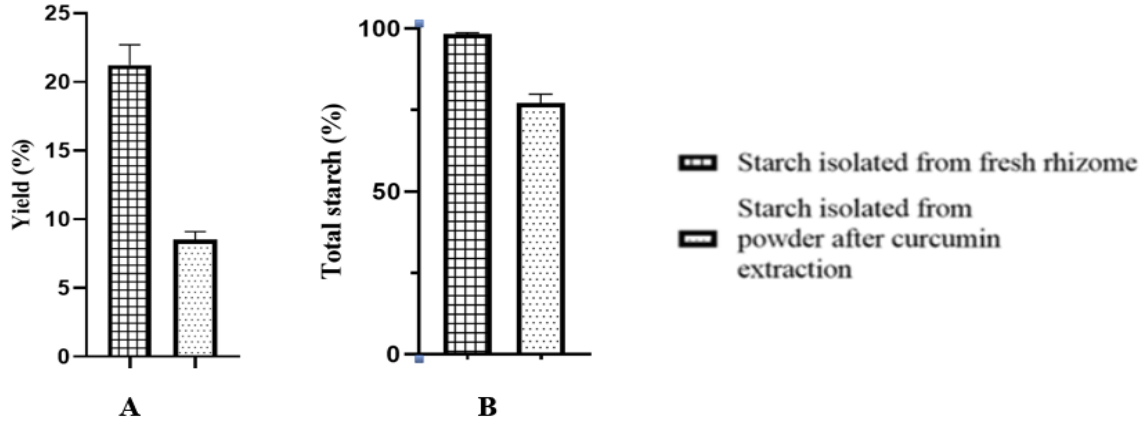

Figure 1. The recovery yield (A) and total starch (B) of starches isolated from the fresh rhizome and from the turmeric extraction residue.

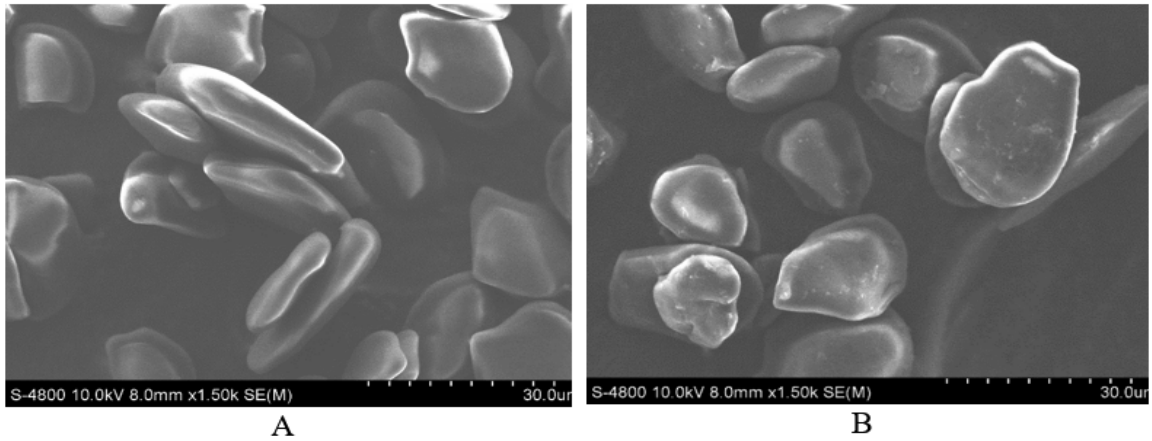

Figure 2. SEM imaging of the Curcuma starches isolated from the fresh rhizome (A) and isolated from the turmeric powder after curcumin extraction, the SEM acquired at the magnification of 1500x.

granule (< 20 µM long) and large (> 20 µM long). The morphology of the starch was consistent with descriptions in earlier studies (Jyothi et al., 2003; Leonel et al., 2003; Pham & Vo, 2017). The starch isolated from the fresh rhizome had a clear, clean surface (Figure 2A) whereas the stained surface (Figure 2B) was observed in the starch isolated from the powder after curcumin extraction. The SEM imaging confirms the difference in purities of starches found in this study.

3.4. Pasting profile of isolated starches

The pasting properties of starches were analyzed and the profiles are presented in Figure 3. The pasting profile of starch isolated from the fresh rhizome was characterized by the pasting properties of a native starch (Figure 3A). The pasting temperature of this starch was 78.3oC and the peak viscosity was reached around 732 cP. The starch paste showed resistance in shear stress since no breakdown was observed during applying the shear rate of 160 rpm at 90°C. When the starch paste was cooled down to 55°C, the viscosity rapidly increased and reached the final viscosity of 1037 cP. This profile of this starch was quite similar to that reported in the literature. Pham & Vo (2017) found that during an analysis of viscosity of Curcuma starch paste, the breakdown was not observed and the final viscosity was also relatively high. A significant change in the pasting profile was recorded in starch isolated from the turmeric powder after curcumin extraction (Figure 3B). Although the pasting temperature negligibly (around 80°C), the peak viscosity was very low (around 106 cP). The low shear stress resistance was recorded since the viscosity was remarkably reduced. The set-

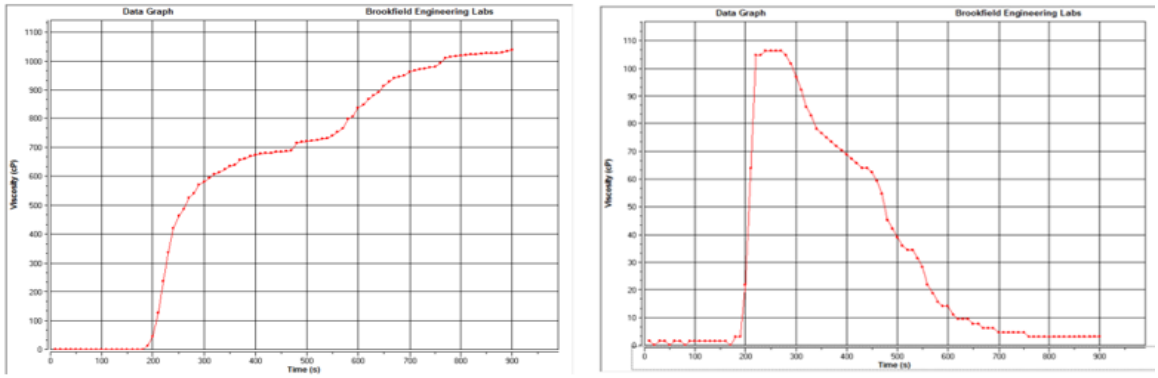

Figure 3. The pasting profiles of starches isolated from the fresh rhizome (A) and the turmeric powder eliminated curcumin (B), measured by Brookfield Engineering Labs viscometer.

back in viscosity during cooling was also not observed. These pasting characteristics indicate that the starch was modified toward annealing as previously mentioned. The annealed starches can keep the pasting temperature but greatly decrease swelling power and final viscosity (Lan et al., 2008; Jayakody et al., 2009).

3.5. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of starches (Figure 4) showed that both starches had the dedicated peaks of starch in the regions of 3000 - 2900 cm^{-1}, 1150 - 1100 cm^{-1} and 1100 - 900 cm^{-1}. The FTIR spectrum peak intensity of starch isolated from the curcumin powder was noticeably reduced compared to that of starch isolated from the fresh rhizome. This finding again confirms for physicochemical change of starch in the powder undergone the extraction of curcumin by using ethanol for 8 h at 50°C. The treatment conditions possibly caused a partial modification of the starch that led to a reduction in the FTIR peak intensities of starch absorptive bands. Xu et al. (2021) observed the partial modification of potato starch in moist-heat treatment at 60°C in 30 mins indicates the absorption at the bands of 1047 cm^{-1} and 995 cm^{-1} were greatly decreased. In this study, the absorbance at 995 cm^{-1} of starch isolated from the curcumin powder after eliminating curcumin reduce from 70.5% to 67.4% compared to that of starch isolated from fresh turmeric. A similar trend was also observed in the absorbance at the band of 1047 cm^{-1} (74.3% to 68.1%). The absorbance at 995 cm^{-1} is considered as bending of C-OH boning, which is responsible for hydroxyl groups of starch whereas the absorbance at 1047 cm^{-1} represents the order structure of the starch (Warren et al., 2016; Xu et al., 2021).

3.6. X-ray diffraction analysis

X-ray diffraction analysis was performed to clarify the long-range structure of isolated starches and the results are presented in Figure 5. The Curcuma starches had a clear B-type crystalline pattern that is dedicated to the starch originating from root or rhizome. The results agreed with previous studies in which the B-type crystalline was found in turmeric starch (Kuttigounder et al., 2011; Pham & Vo, 2017). Although different in the peak intensity, the peaks were well pronounced at 6.3, 9.6, 11.9, 14.5, 17.1 and 18.7 Å in two obtained starches. The peak at 11.9 Å had the highest intensity followed by a peak at 17.1 and 18.7 Å. The finding in this study is different from what was reported in a study by Kuttigounder et al. (2011) in which the peak at 17 Å was recorded with the highest intensity. The difference in the most pronounced peak would be due to the difference in origin of starches. The relative crystallinities of starches isolated from the fresh rhizome and the turmeric powder after extracting curcumin were 26.8 and 20.4%, respectively. The crystallinity of starches obtained from this study was in the reported range of 15 to 45% (Zobel, 1988). The starch isolated from the turmeric powder after curcumin extracting, as aforementioned, was partially modified and

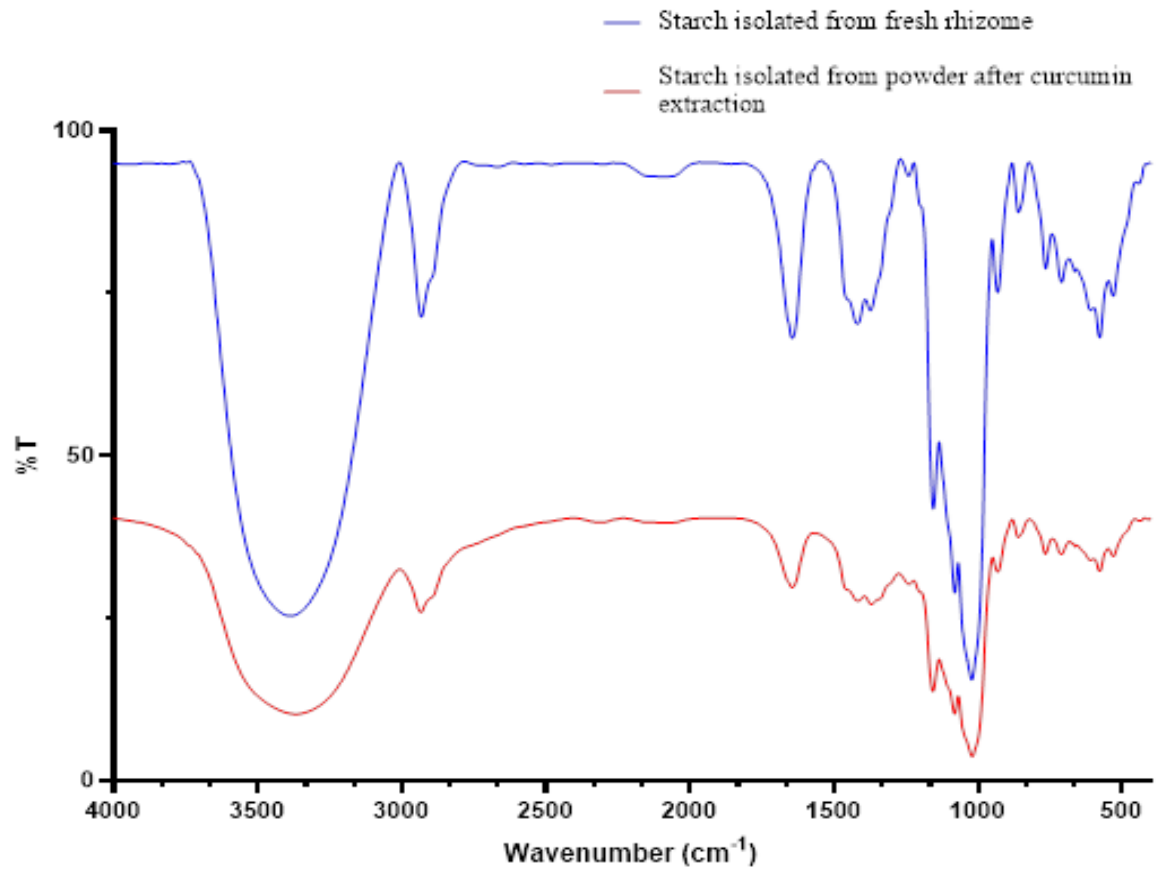

Figure 4. FTIR spectra of starches isolated from the fresh rhizome and the turmeric powder after extracting curcumin.

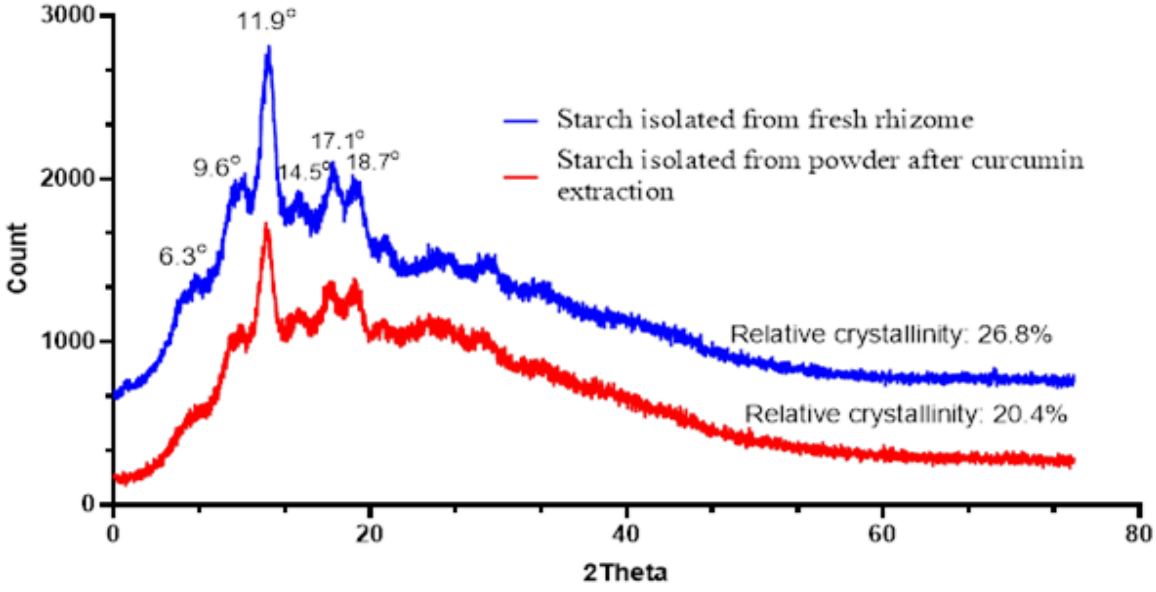

Figure 5. X-ray diffractograms of isolated starches from two different methods, data were offset for clarity.

thus likely led to a reduction in the relative crystallinity.

3.7. Curcumin analysis

The curcumin in turmeric powder and starches were quantified by using RP-HPLC-PDA method. The chromatograms and the curcumin results are presented in Figure 6 and Table 2, respectively. Previous studies demonstrate that curcumin exists in turmeric with other curcuminoids. The RP-HPLC-PDA elution of curcuminoids was in order as bisdimethoxy curcumin, bisdimethoxy curcumin and curcumin (Gugulothu et al., 2012; Peram et al., 2017). In the present study, bisdimethoxy curcumin, dimethoxy curcumin and curcumin were orderly eluted at 10, 10.84 and 11.756 min (Figure 6). The curcumin was predominant over other curcuminoids with the total peak area accounting for over 85%. Quantitative analysis showed that the powder contained 3.746 mg/100 g (dw) curcumin. The level of curcumin in turmeric (Curcuma longa) was higher than the level documented. Two varieties of turmeric in Vietnam analyzed before contained from 2.977 to 3.198 mg/100 g (Hayakawa et al., 2011). According to this study, the level of curcumin from Vietnamese turmeric was higher than that of varieties collected from Thailand, Japan, and Indonesia. The residues of curcumin in the isolated starches were very low ranged from 18.4 to 66.5 mg/100 g (dw) (Table 2). The starch isolated from the conventional method of using fresh turmeric had a very low curcumin level (18.4 mg/100 g), indicating the limited application based on the biological curcumin in the starch. The curcumin remained in the starch isolated after ethanol extracting was 66.5 mg/100 g, suggest for the effectiveness of using ethanol to extract the curcumin since over 98% of curcumin was extracted.

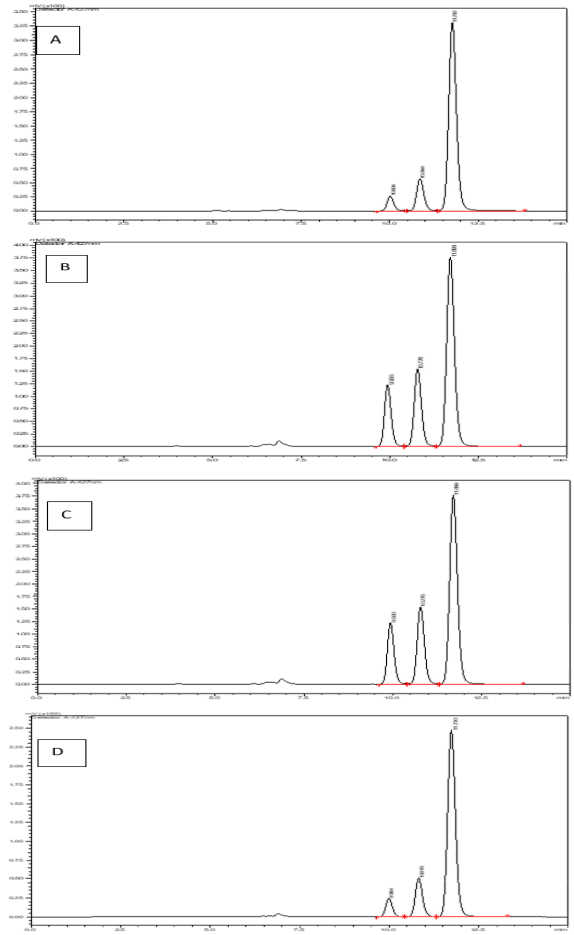

Figure 6. Chromatograms of (A) curcumin standard, (B) in turmeric powder, (C) in starch isolated from the fresh rhizome, and (D) in starch isolated from the turmeric powder after eliminating curcumin by ethanols, the analysis using RP-HPLC-PDA.

Table 2. The blue value of starches isolated from the fresh rhizome and turmeric powder

Sample	Curcumin content (mg/100 g)
Turmeric powder	3,746.5 ± 141.5[a]
Starch isolated from fresh rhizome	18.4 ± 0.9[b]
Starch isolated from the turmeric powder after curcumin extraction	66.5 ± 3.6[c]

Values are expressed as mean ± SD of triplicates.
Values followed by different lowercase letters in superscripts were significantly different at $P < 0.05$.

4. Conclusions

The starch was initially recovered from the turmeric powder after curcumin extraction by using ethanol for 8 h at 50°C. The obtained starch had a moderate yield of extraction and purity. The physicochemical properties such as iodine binding capacity, SEM imaging, pasting properties, FTIR and X-ray diffraction were analyzed. The results showed that the starch was partially modified. Quantitative analysis proved that ethanol extraction effectively removed curcumin from the starch powder, only around 0.5% curcumin residue in the isolated starch. Modifications should be implemented to improve the pro-

cedure of starch recovery from the organic waste originating from the curcumin extraction process.

Acknowledgment

The authors declare no conflicts of interest.

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