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Extraction and characterization of Tikhur starch (*Curcuma angustifolia*) and its utilisation in the development of edible films

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ABSTRACT

Isolation and characterization of starch from *Curcuma angustifolia* rhizome (Tikhur) was carried out for the development of novel edible food packaging film.Various characteristics viz. physicochemical and functional was studied for determining its suitability for edible film development. The low percentage of ash, protein and fat revealed the purity and high quality of isolated starch. Functional characteristics indicated good paste clarity and water retaining capacity. The isolated starch contains 31.9 ± 0.45% amylose which offer better stability and film forming property. Isolated starch was used for the development of edible food packaging film by solvent casting method and its mechanical, optical, barrier, thermal, and morphological properties were examined. The developed starch film displayed good tensile strength and flexibility. The characteristic diffraction peak of the starch film showed more crystalline structure than the amorphous structure. The thermogravimetric investigation revealed the required thermal stability of extracted starch film for the packaging of food products with 40% of the mass loss occurred between 330 °C and 410 °C. The film was uniform, transparent and easy to handle. The study showed that *Curcuma angustifolia* rhizome starch is a sustainable starch source for application of starch in food packaging industry.

Key words : *Curcuma angustifolia, Starch, Functional characteristics, Solvent casting method, Edible food packaging film*

Introduction

Due to the increasing amount of non-biodegradable waste being generated and the problems associated with recycling the majority of currently available synthetic packaging, there has been increased attention to the development of novel biodegradable and edible food packaging films. Biodegradable films are now utilising ingredients from agriculturally derived products to minimise the environmental effects of non-biodegradable petroleum packaging. Various biopolymers and their combinations are used for edible or biodegradable films formation (Fakhouri *et al.,* 2019; Gutiérrez *et al.,* 2015; Shokri *et al.,* 2020). One of the most favorable polymer materials with a film-forming capability and abundant availability is starch (Cheng *et al.,* 2021; Cheng *et al.,* 2019; Tavares *et al.,* 2019). It has the ability to form a matrix that is continuous, less expensive, edible, and renewable. Recently, several publications have discussed the usage of starches taken from various sources in order to make films and coatings. Recent

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research has also drawn attention to the potential therapeutic qualities of starchy products, which are now considered strategic materials for the future. Because industrial conventional starches such as corn, wheat, and potatoes may become expensive due to population growth, the search for alternative sources of starch has been encouraged. After grains and legumes, rhizomes and tubers are the third most important food crop, with starch as a key component (Daiuto *et al.,* 2005; Sukhija *et al.*, 2016b). Tropical countries like India have a great variety of roots and tubers (Peroni *et al*., 2006).Extensive research has been conducted to investigate the sustainable commercial use of unconventional starches as a matrix in the creation of edible films.

Curcuma angustifolia is a member of the *Zingiberaceae* family, which includes plants such as ginger and turmeric. It has around 50 types and 1000 species and is found in the tropics of Australia, Africa and Asia. It is one of the lesser-known and valuable sources of starch (Franklin *et al., 2017*). Its starch is used in a variety of foods because of its thickening, gelling, and water-retaining properties (Kumari *et al.*, 2017; Raj *et al.*, 2018). Its rhizome is a major ingredient, easily digestible, and valued article in the diet which has not yet been fully investigated and utilised for industrial purposes.

Curcuma angustifolia, widely known as "East Indian arrowroot" or "white turmeric,", comes out as a source of unconventional starch. However, the structural and physicochemical characteristics as well as their extraction process have not been studied extensively. Because of easy extraction process, it has really been a cost-effective source of starch extraction. It contain moderate amount of amylose and is ideal for the development of films with strong functional qualities (Das *et al.,* 2015; Rani and Chawhaan, 2012). Since the starch of *Curcuma angustifolia* has received little attention, despite its potential application in the food industry. There are several reasons why such unconventional starches might be explored, including the potential for monetizing underutilised starches. Thus, work was done for the value addition of such an unconventional source of starch in the market. Characterization of starch is done to determine its suitability for industrial applications such as edible packaging films with the required performance. Developed film was further characterized based on its mechanical, optical, barrier, thermal, and morphological properties.

Materials and Methods

Starch Extraction

Curcuma angustifolia (Tikhur) rhizomes for the harvest year 2020 were acquired from Baster, Chhattisgarh state of India. Starch from the rhizome was isolated using wet processing with modifications from (Patel *et al*., 2015).

Characterization of Starch

Physical Properties

Starch Yield

The percentage yield of starch was determined based on the following equation

$$\text{Starch yield (\%)} = \frac{\text{Weight of starch}}{\text{Weight of rhizome}} \times 100 \qquad (1)$$

Bulk Density

The bulk density of starch was determined by using bulk density apparatus (220V, "Harrison's, Pharma Machinery, New Delhi, India"). The tapped density was determined by taking the ratio of mass versus volume of the tapped sample (Franklin *et al.,* 2017).

$$\text{Bulk density/Tapped density (g/ml)} = \frac{\text{Mass of starch, g}}{\text{Volume of starch, ml}} \quad .. (2)$$

True Density

True density was determined by using solvent displacement method (Raj *et al.,* 2018) using toluene as a solvent.

$$\text{True density (g/ml)} = \frac{\text{Mass of starch, g}}{\text{Volume of starch, ml}} \quad .. (3)$$

Porosity

The porosity of a material is the proportion of air between particles compared to the volume of particles

$$\text{Porosity (\%)} = \frac{\text{True density} - \text{Bulk density}}{\text{True density}} \times 100 \quad .. (4)$$

Chemical properties

Moisture content

Moisture content was determined gravimetrically using the hot air oven following the standard procedure described by AOAC, 2005.

$$\text{Moisture content \% (wb)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad .. (5)$$

Determination of pH

With the use of a digital pH meter, the pH of an aqueous starch suspension at 1% (w/v) was determined (Das *et al.*, 2015).

Determination of Ash Content

An ash content test was conducted by using the muffle furnace following the standard procedure described by AOAC, 2005.

$$\text{Total ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100 \quad .. (6)$$

Crude protein content

The crude protein content of starch was measured by using the Kjeldahl apparatus following the standard procedure described by AOAC, 2005.

Calculations

$$\frac{N}{\frac{g}{kg}} = \frac{(\text{mL Hcl of sample} - \text{mL Hcl of blank}) \times \text{normality} \times 1.401 \text{ final volume}}{\text{Weight (g)} \times \text{aliquot volume (ml)}} \quad .. (7)$$

Crude fat content (Ether extract)

Crude fat content (ether extract) was extracted using the Soxhlet apparatus following the standard procedure described by AOAC, 2005.

$$\text{Fat content \%} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100 \quad .. (8)$$

Amylose content

Colourimetric analysis was used to measure amylose content, by transmitting light through a coloured complex of amylose with iodineat 625nm using UV-VIS spectrophotometer, using the methodology described in (Ambardekar *et al.*, 2011).

$$\text{Amylose \%} = \frac{\text{Absorbance} \times 100}{2 \times \text{g solution} \times \text{mg starch}} \times 100 \times 28.414 \quad .. (9)$$

Functional Properties

Water/oil retaining capacity (WRC/ORC)

Water/oil retaining capacity were determined according to (Anderson *et al.*, 1970) with slight modifications.

$$\text{WRC/ORC} \quad .. (10)$$

Water Solubility Index (WSI)

Water Solubility Index was determined by using the methodolology described by (Anderson *et al.*, 1970) with minor modifications.

$$\text{Solubility (\%)} = \frac{\text{Dry supernatant (g)}}{\text{dry starch (g)}} \times 100 \quad .. (11)$$

$$\text{Swelling power} \left(\frac{g}{g}\right) = \frac{\text{Wet starch (g)}}{dry\ starch\ (g) - dry\ supernatant} \quad .. (12)$$

$$\text{WSI} \left(\frac{g}{g}\right) = \frac{g\ of\ soluble\ starch}{g\ of\ original\ starch} \quad .. (13)$$

Paste clarity

UV-VIS spectrophotometer was used to determine the starch paste clarity at 640 nm using distilled water as a blank (Liu *et al.*, 2019).

Design of experimental procedures for film-forming solution

Preparation of the film was done by the solvent casting method, partly adopted from Ghanbarzadeh *et al.*, (2010). A filmogenic solution was prepared using extracted *Curcuma angustifolia* (Tikhur) starch, which was used as a matrix. The plasticizer was glycerol, while the solvent was distilled water. Pre-weighed *Curcuma angustifolia* (4.0 g per 100 ml) was stirred with distilled water at 500 rpm using a mechanical stirrer for 15 min. Glycerol was added to the starch solution while being heated to a temperature of 80 °C and mechanically stirred for 30 min at 500 rpm. Prepared solution was poured into the casting plate and dried at 40 °C for 16 h in an oven.

Characterization of Curcuma angustifolia (Tikhur) starch films

Mechanical properties

The determination of mechanical properties of films (9 cm x 2 cm) was carried out on a texture analyzer (Stable microsystem with exponent Software, U.K.), according to ASTM D882-12. Force in Newton and deformation in millimetres were measured during extension at 50 mm min-1.

The tensile strength in MPa was calculated as

$$TS = \frac{F}{L \times W} \quad .. (14)$$

Where F represents the maximum tensile force

when the film breaks, film's thickness is measured as L, and a film's width is measured as W

EAB % was calculated as

$$EAB\% \quad \frac{Li–Lo}{L0} \times 100 \qquad .. (15)$$

where L_0 and L_i, represents intial and final elongation respectively.

Physico-chemical properties

Thikness

A digital micrometer was used for determining the thickness of the developed films by taking an average of measurement at three different positions.

Moisture content

Preconditioned (23% RH, 25 °C) film samples (2 cm^2) were put in oven at 100 ± 5 °C and dried until a constant weight was obtained.

$$\text{Moisture content} = \frac{Mi - Mfi}{Mi} \times 100 \qquad .. (16)$$

In this equation, Mi and Mfi refer to the initial and final weight of the samples, respectively.

Moisture absorption

Dried film samples (2cm^2) were conditioned to 0% RH using a saturated solution of $CaCl_2$ for 24 h and weighed. Dried film samples were conditioned to 75% RH containing a saturated solution of NaCl. The weighed film samples were assessed every hour until equilibrium was reached. The following equation was used to calculate moisture absorption

$$MA\ (\%) \quad \frac{Ws - Wo}{Wo} \times 100 \qquad .. (17)$$

Where Ws represents the weight of the film in 75% RH at time t and Wo represents the initial dry weight of the film at 0% RH, respectively.

Water vapour permeability (WVP)

Testing for water vapour permeability (WVP) was carried out at room temperature with some modifications to the ASTM E96/ASTM E96M-16 method. Permeation cells for WVTR were small vials with a depth of 4.5 cm and a diameter of 2.5 cm (Dashipour *et al.*, 2015).

The water vapour transmission WVTR (g/m^2h) and water vapour permeabilityWVP (g m^{-1} $h^{-1}$$KPa^{-1}$) were determined as:

$$WVTR = \frac{Slope}{\text{Film transfer area}} \qquad .. (18)$$

$$WVP = [WVTR/P\ (R1–R2)] \times T \qquad .. (19)$$

P is the saturated water vapour pressure at test temperature (25°C), R1 is the relative humidity in a desiccator (75%), R2 is the relative humidity in the vial (0%), and t is the film thickness in millimeters.

Water solubility (WS)

Film samples (4 cm^2) were conditioned at 0% RH using anhydrous calcium chloride as desiccant till the constant weight was obtained and weighed (W1). Conditioned film samples were immersed into 50 mL of distilled water for 24 h at 4, 25, and 50 °C ± 0.5 °C in a shaker at 150 rpm followed by filtration with Whatman No.1 filter paper (previously dried to constant weight). Film residues were collected and re-dried to produce a final constant weight, which was used to calculate dry matter (W2) weights.

$$WS\ \% = \frac{W1 - W2}{W1} \times 100 \qquad .. (20)$$

Internal transmission and opacity

UV-visible spectrophotometer (*UV2250, Shimadzu*) was used to determine the internal light transmittance and opacity of the developed film sample.

$$\text{Opacity} = \frac{Abs\ 600}{X} \qquad .. (21)$$

Where X represents the film thickness in (mm). The transmittance values were converted to absorbance using Beer-Lambert Law.

Morphological characteristics

Filed emission scanning electron microscopy observations (*Gemini 300, Carl Zeiss, Japan*) were made for determining the morphological characteristics of the developed films. Film samples (1 cm^2) were fixed on FESEM stubs. It was covered with carbon adhesive tape double-sided with a gold layer in an ion sputter and scanned at an acceleration voltage of 3kV.

Functioanl group analysis

Functioanl group analysis of developed starch film was carried out using Fourier transform infrared spectroscopy. FTIR spectra were obtained from (*Bruker, ATR-FTIR*) with a diamond crystal. Films

samples (3cm^2) were scanned using ATR mode in the range of 4000-700 cm^{-1} with a 4 cm^{-1} spectral resolution with 32 scans per sample.

Crystallinity analysis

With the help of X-ray diffraction (*Smartlab, Rigaku Technologies, Japan*), the crystalline nature of the films was determined at a step width of 1.2°/min/ 0.05°min^{-1} over the 2θ between 5 to 40° using curved graphite crystal monochromator and Ni-filtered Cu K α radiation (λ= 0.1541 nm).

Thermal properties

The thermal properties of the film sample was obtained in an (*EXSTAR TG/DTA, model 6300*). A sample weighing about 10 mg was heated from 35°C to 650 °C at a rate of 10 °C. min^{-1}. Under nitrogen, the measurements were conducted at a flow rate of 100 ml/min^{-1} while alumina powder was used as a reference.

Results and Discussion

Characterization of Starch

Physical properties

Extraction of *Curcuma angustifolia* rhizome resulted in a white coloured odourless starch, with a yield of 13.79 ± 2.75% on a dry weight basis, which was in line with the results given by (Patel *et al.,* 2015; Raj *et al.,* 2018). While determining the true density of *Curcuma angustifolia* (Tikhur) starch particles using the solvent displacement method, the void space and pores were filled with toluene, resulting in a higher true density than bulk density and tapped density. Porosity is a measure of the amount of air between particles in relation to the volume of particles. If starch present higher porosity then resultedstarch films also present higher porosity and roughness. WVP of starch films is also influenced by the porosity of the starch as in pinhao flour film (Daudt *et al.,* 2016).

Chemical properties

The moisture content of extracted *Curcuma angustifolia* starch on a wet basis was calculated as 5.97±0.43%. Starches with lower moisture content can be stored for a longer period. The pH of the extracted starch was near to neutral. The low percentage of ash, protein and fat revealed the purity and high quality of extracted starch from rhizomes which does not significantly affect the functional properties of root and tuber starch compared to cereal starch due to its low content (Peroni *et al.,* 2006). In addition to proximate components, amylose percentage also play a significant effect on the chemical properties of starch which help in determining its applications. Amylose content affects starch , swelling power, gelatinisation and retrogradation properties. Because high amylose starch permits a stronger association of the amylopectin within and with amylose which resulted in increased resistance of starch granule to swell and gelatinized. The isolated starch contains 31.9 ± 0.45% amylose which coincides with the result suggested by (Franklin *et al.,* 2017) and (Rani and Chawhaan, 2012). Intermediate amylose

Table 1 A: Physical, Chemical & Functional Properties of Extracted Starch

	Properties	Units	Results
Physical properties	Starch Yield	%	13.79 ± 2.75%
	Bulk Density& Tapped density	g/ml	0.814 ± 0.04 & 0.643 ± 0.042
	True Density	g/ml	1.688 ± 0.06
	Porosity	%	57.16 ± 0.91
Chemical Properties	Moisture content	%	5.97 ± 0.43
	pH	-	7.1 ± 0.15
	Ash Content	%	0.52 ± 0.06
	Protein	%	0.92 ± 0.16
	Fat	%	0.54 ± 0.08
	Amylose	%	31.9 ± 0.45
Functional Properties	(WRC/ORC)	g/g	2.32 + 0.18 g/g and 1.92 + 0.04
	Solubility at 90°C	%	4.69 ± 0.02
	Swelling power (SP) at 90°C	g/g	3.21 ± 0.01
	Water solubility index (WSI)	g/g	0.010 ± 0.001
	Paste Clarity at Day 1	g/g	90.89 ± 0.02

Table 1 B: Functional Properties of Extracted Starch at different temperatures.

Parameters	50 °C	60 °C	70 °C	80 °C	90 °C
Solubility,%	2.87 ± 0.05	2.99 ± 0.08	3.15 ± 0.01	3.68 ± 0.10	4.69 ± 0.02
Swelling power, g/g	2.54 ± 0.04	2.72 ± 0.01	2.79 ± 0.02	2.84 ± 0.03	3.21 ± 0.01

Table 1 C: Paste of Extracted Starch during storage.

Parameter	Day 1	Day2	Day3	Day4	Day5
Paste clarity	90.89 ± 0.02	68.01 ± 0.15	50.02 ± 0.12	46.85 ± 0.01	44.72 ± 0.18

Data were means ± standard deviations, n =3.

content of *Curcuma angustifolia* starch offer better stability and viscoelasticity for the development of biodegradable films.

Functional properties

Starch granules have a water and oil retaining capacity based on their tendency to absorb water and oil, as well as the degree to which water molecules adhere to the starch granules. The amount of excess water taken up by the starch after swelling is equal to the starch's integrity in aqueous solutions and the amount of gel formed (Nafchi *et al.,* 2013). The swelling power and solubility of starch depends on associative forces between starch chains in crystalline and amorphous domains. *Curcuma angustifolia* (Tikhur) starch showed low solubility ranging from 2.87 ± 0.05 to 4.69 ± 0.02 % from 50-90 °C as compared to cereal starches. It can be attributed to the complexing of amylose with lipids and the high stability of starch amylopectin structures, which prevented it from dissolving in solution during heating (Zhang *et al.,* 2017). The clarity of starch paste is related to the dispersion of starch in water. It is also related to the retrogradation of the starch, as retrogradation leads to an increase in turbidity and lowers the transmittance. Paste clarity of extracted starch decreased gradually during storage which may be due to recrystallization of starch (Hoover, 2001). The paste clarity is directly correlated with the colour and transparency of the films developed from this starch.

Table 2. Physico-chemical and Mechanical Properties of Film

Particulars	Results
Thickness, mm	0.17 ± 0.013
Tensile strength(MPa)	6.15 ±0.59
Elongation at break (%)	58.87 ± 1.34
Moisture Content (%)	9.32 ±0.79
Moisture absorption (%)	9.71 ± 0.08
WVP, g.mm./k.Pa. h. m2	1.15 ± 0.12
Water Solubility (WS %)	
4 °C	6.56 ± 0.86
25°C	9.26 ± 0.99
50°C	10.81 ± 1.58
Internal transmission	
Tr280 (%)	15.56 ± 0.35
Tr600 (%)	44.61 ± 0.07
Opacity	2.06 ± 0.06

Data were means ± standard deviations, n =3.

Characterization of Film

Physico-chemical and Mechanical Properties

Curcuma angustifolia (Tikhur) starch film presented a homogenous surface with no cracks. Film was easily detached without tearing and was easy to handle. The results of thickness, TS, EAB, MC, MA and WVP are shown in Table 2. The film was uniform in thickness with an average thickness, from 0.17 to 0.183 mm. As compared with starch-based films, the developed film had a low moisture content (Chandla and Saxena, 2017), (Sindhu and Khatkar, 2018), and (Pelissari *et al.*, 2013). The tensile strength was also higher (Chandla and Saxena, 2017). Elongation at break represents the film's stretchability and flexibility. The WVP of the *Curcuma angustifolia*(Tikhur) starch film was close to that of the potato starch-based film (Dharmalinga Jha, Pankajm *et al.,* 2020). The film solubility in water was increased with an increase in temperature and ranged from 6.56 ± 0.86 to 10.81 ± 1.58%. Curcuma angustifolia (Tikhur) starch film was less soluble than arrowroot (*Maraanta arundinaceae* L.) starch films which presented solubility varying from 6.46 ± 0.67 to 16.71 ± 1.23% (Nogueira *et al.*, 2018). Light transmittance of *Curcuma angustifolia* (Tikhur) starch

films through UV region (280 nm) was 15.56% representing its moderate UV light barrier properties which was lower than lotus starch films (25.6% to 28.1%) reported by Sukhija *et al.,* (2016a). However, light transmittance in visible region was 44.61%. As lower levels of light transmittance resulted in higher opacity values which may be due to presence of more crystalline zone as reported by Sun *et al.*, (2014).

Morphological characteristics

The morphology structure of the edible film is a very important characteristic because many properties of the film, such as mechanical, thermal, and barrier, depend on it. FESEM micrographs at 50,000 and 60,000 magnification of the film surface are shown in Fig. 1. The starch film displayed a smooth, continuous, and uniform surface without any visible cracks or pores.

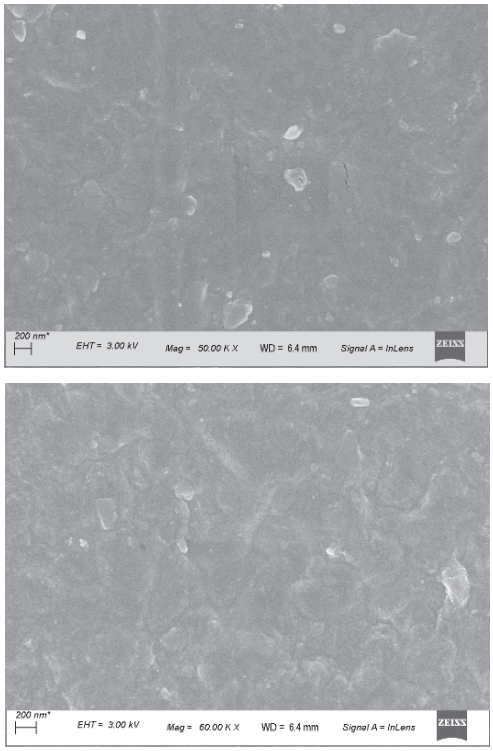

Fig. 1. FESEM micrographs of *Curcuma angustifolia* (Tikhur) starch film at 50,000 and 60,000 x magnification

Functional group analysis

The FTIR spectra of *Curcuma angustifolia* (Tikhur) starch film is shown in Fig. 2 which was used to confirm the surface functional groups. Peak at 2895 $cm^{-1}$was assigned to C–H stretching. While the peakat 3362 and 3523, represent the stretching of O–H group. In the region around 1636 cm^{-1}, there is a peak that is closely associated with presence of water molecules associated with the starch (Wijaya *et al.,* 2019). Protein traces in the extracted starch may also be responsible for the presence of this group in developed starch film (Franklin *et al.,* 2017).

Crystallinity analysis

The characteristic diffraction peak showed that the crystalline structure of isolated starch film was more dominant than the amorphous structure. Several distinctive peakswere observed in the XRD pattern (2θ around 5.51°, 17.02°, 19.81°, and 21.94°) with percentage crystallite index of 56.23. Normally, starch crystals are type V, which results from the complexation of glycerol and amylose. The peak at 17.02° could be associated to amylose, while the peak at 21.94° to Vh crystallinity (Dai *et al.,* 2019; Huang *et al.,* 2020). The crystallinity of starch films could be due to amylose crystallisation that occurs early in the film formation process, as well as the slow crystallisation of amylopectin that occurs during storage.

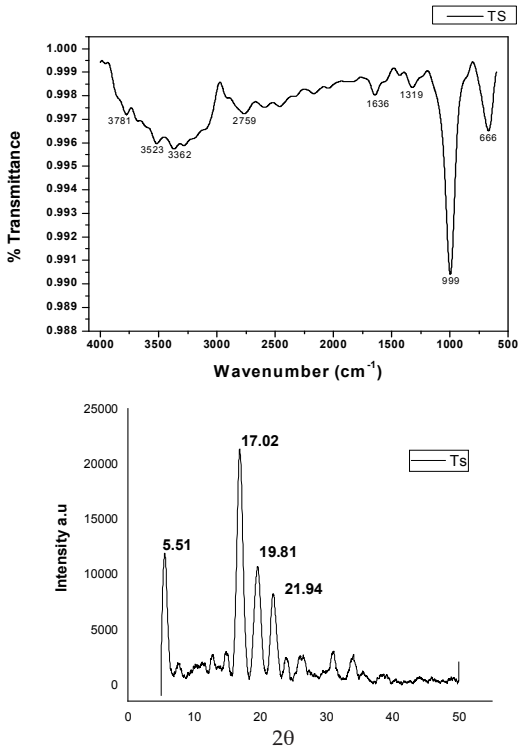

Fig. 2. A: FTIR spectra B:XRD analysis of *Curcuma angustifolia* (Tikhur) starch film

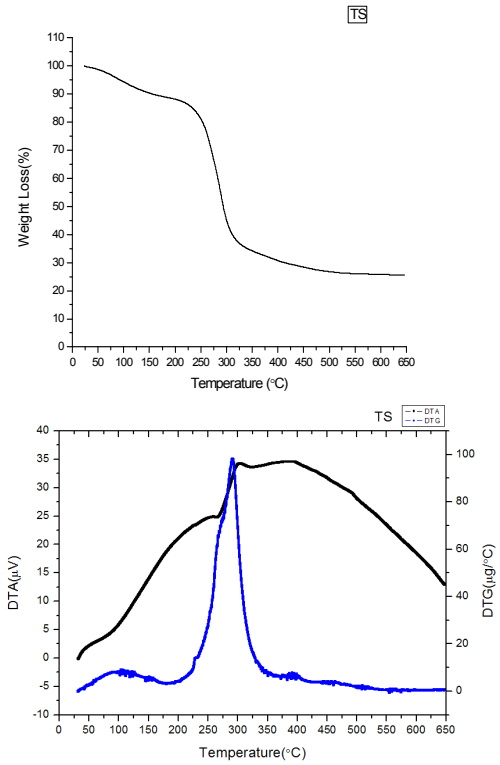

Fig. 3. TGA and DTA/ DTG curvesof Curcuma angustifolia (Tikhur) starch film

Thermal properties

Curcuma angustifolia starch film had three phases of mass loss between temperature ranges of 25–600 °C. The first phase of mass loss (16%) corresponded to evaporation of water and plasticizer from the film sample in the temperature range of 25 to 200 °C (Franklin *et al.*, 2017). The second phase of major mass loss (54.5%) that occurred between 200 and 340°C was due to the polymer chain decomposition of starch. The third stage (350–650 °C) weight loss (8.9%) was due to disintegrated residue generated from the oxidative atmosphere process. Carbonization occurred at temperatures above 550°C. The DTG curve shows decomposition peaks at 290 °C, showing the depolymerization of the starch polymer chain. TGA results revealed the required thermal stability of isolated starch film for the packaging of food products.

Conclusion

Curcuma angustifolia (Tikhur) starch is a source of unconventional starch which was isolated and characterised. Rhizomes of *Curcuma angustifolia* was used for starch isolation by wet milling method. Characterization of isolated starch was carried out based on its physico-chemical and functional properties. Isolated and characterized starch was used for development of edible food packaging film by solvent casting method. Despite their hygroscopic nature, extracted starch film exhibited good water vapour barrier properties. Developed film had uniform and smoother structure, which provided a solid foundation for preventing water vapour migration. Edible starch film was transparent and had a good tensile strength. It has a potential to arise as an unconventional source of starch in food and food packaging industries.

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