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Evaluation of properties of native and modified phyto-polymer, starches of *Curcuma angustifolia* and development of polymeric films

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In the present study, polysaccharide- starch of *Curcuma angustifolia* was isolated and modified by chemical reactions- acetylation, hydroxy-propylation and carboxy-methylation. The acetyl, hydroxypropyl, carboxylmethyl groups percentage and degree of substitution (DS) for acetylated, hydroxypropylated and caboxymethylated starches of *C. angustifolia* were determined 6.89%, 0.28%, 0.06%, and 0.04, 1.30 ,0.75, respectively. The introduction of carboxymethyl group significantly increase the swelling power and solubility while gelatinization temperature was reduced to 68.70^{0}C in modified starch in comparison to native (97.03^{0}C) starch of *C. angustifolia*. Similarly, examination of water absorption capacity showed increasing trend in modified starches. The suspensions from native and modified starches were prepared and polymeric films were developed utilizing glycerol as plasticizer. The casted films thickness varied between 0.040-0.160 mm. The mechanical properties of different polymeric blends showed wide variation (81.1-427.5 kgf/cm^2). The maximum tensile strength showed in acetylated modified blend (427.5 kgf/cm^2) and PVOH blend *C. angustifolia* (391.79 kgf/cm^2) while the polymeric film blend with native potato starch, prepared for comparison, showed minimum tensile strength (81.1 kgf/cm^2).

Keywords: *Curcuma angustifolia,* Starch, Modified starches, Acetylation, Hydroxypropylation, Carboxymethylation, Polymeric films, Tensile strength.

INTRODUCTION

Synthetic non-degradable petrochemical-based polymers poses one of the greatest threats to ecology today as they remain more or less unchanged in landfills even after a period of five decades. This alarming situation necessitates the investigations of viable alternatives or complements to synthetic petro- based chemicals with the aim to reduce their applications. Polysaccharides, i.e., starch and cellulose are the potential renewable raw material, available in plants as stored food, abundantly distributed in forests and can be converted into biodegradable eco-friendly products products by some chemical modification or processing directly [1]. Various work has been done on bioplastic synthesis by cassava, corn, sugar palm, jack fruit seed starch, potato, pea, wheat gluten, tamarind seed and banana peel [2-10].

Curcuma angustifolia Roxb. ('Tikhur) belongs to the family of Zingiberaceae. It occurs in central India, West Bengal, Madras provenances and lower parts of Himalayan ranges as associate of *Shorea robusta*. The plant resembles to *C. Longa* with stout root stock, globose tubers at the ends of the fibrous roots. The rhizomes are collected by the tribals in winter season and used for extraction of starch for edible purposes. In this study, for the first time the modification of *C. angustifolia* starch through introduction of acetyl, hydroxypropyl and carboxymethyl groups was undertaken and utilized in the development of ecofriendly films.

MATERIAL AND METHODS

Isolation and purification of polysaccharides.

The tubers of *C. angustifolia* species were collected from the Achanakmar, Dhamtari forest area in Chattisgargh and extracted by a standard method [11]. Acetylation, hydroxypropylation and carboxymethylation products of different starches were prepared and the degree of substitution was determined according to standard procedure [12, 13]. Physico-chemical properties of native and modified starches were assessed as follows.

Color, pH value and density measurement

Color of the starches was determined by visual color appearance. A 20 % w/v dispersion of the native and modified starches was shaken in water for 5 min and pH of water phase was determined using a digital pH meter. The density of starches from different species was determined by the specific gravity method and calculated by using the formula:

Density (g/ml)= W1 /[(W1 +W2) – W3)] D1

where, W1 =weight of starch, D1 = density of

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xylene, W2 = weight of bottle with xylene, W3 = weight of bottle with starch.

Swelling power, solubility and water absorption capacity (WBC)

The swelling power and solubility of starches were determined according to the standard method [14]. 0.5g of starch was taken in 20 ml of distilled water in pre-weighed centrifuge tubes and content was kept on water bath with shaking for half an hour at different temperatures, i.e., 60, 70, 80, and 90°C. The supernatant was collected and poured into a dish to analyze solubility and put in an oven at 130°C. The moisture content in the precipitate was assessed as follows: 1 g sample was dissolved in 10ml solvent (water, dimethyl sulfoxide (DMSO), chloroform, toluene, acetic acid, ethanol, ethyl acetate, propanol-2, butanol, diethyl ether, acetonitrile, 1N alkali, 1N acid, tetrahydrofuran (THF) and the solubility pattern was assessed. The WAC of each native and modified starch was determined by dissolving 1 g starch in 10 ml distilled water and centrifuged at 2000 rpm for 10 min. The supernatant was decanted and the final sample weight (Wf) was determined. The WAC (expressed in grams of H_2O absorbed per gram) was calculated after centrifugation [14]:

$$WBC = (Wo - Wf) / Wo$$

where: Wo = sample weight (g), Wf = final sample weight after centrifugation (g).

Gelatinization temperature was determined with the help of a microscope using Congo red dye [15].

Stability and clarity of starch pastes

Stability and clarity of native and modified starch pastes were determined at 4^0C; 0.2 g of starch sample were taken in screw cap-tubes with 5 ml of water and kept in a boiling water bath for 30 min. The % T (Transmittance) was determined at 650 nm against a water blank on a spectrophotometer after cooling to room temperature, and was recorded after 24, 48 and 72 h [16].

Microscopy

A starch dispersion was stirred using a wire loop and was transferred onto a microscope slide. Leica-1000× advanced research scanning electron microscope (SEM) facility was availed from Sophisticated test and Instrumentation Centre, Cochin University, Kochi, Kerala).

Development of polyfilms

Filmogenic suspensions of unmodified and acetylated, hydroxypropylated and carboxymethylated starches of *C. angustifolia* were used for film development adding glycerol as plasticizer (10:1). The starch:water (1:20) suspension was gelatinized. After completion of gelatinization, plastisizer and other additives (10:1) were added under constant stirring for 10 minutes. The additives chitosan, bentonite, mucilage, lignin extract and polyvinyl alcohol were used to check the compatibility. The films were prepared by a casting technique, 20 g suspensions were poured into 15 cm petri dishes, dried at room temperature and cured in an oven at 100^0C to constant weight.

Thickness and film appearance

Thickness of the films was determined using a digital coating thickness gauge. Randomly ten measurements were taken at different points of the film specimen. Homogeneity and appearance of the films were examined by visual observation and by SEM with a JEOL JSM 6360 electron microscope (Japan). The film solubility and opacity were determined [17].

Mechanical properties

The tensile strength of polymeric films (sized 12 × 2 cm^2) was measured by using an Instron Universal Testing machine by adopting IS 2508 method.

Statistical analysis

The results were statistically analyzed using SPSS Version 14 (SPSS Inc., Chicago, III., USA)

RESULTS AND DISCUSSION

Total starch content of *C. angustifolia* was estimated as 38.11% and the color of the starch was white. Variation in starch shapes and size is shown in SEM (Fig.5). The shapes of *C. angustifolia* starch were rounded, oval to elliptical, round, oblong, triangular and asymmetrical, granule length varied between 16.36-48.18 µm (mean 34.62 µm) and width between 20.90 to 40.91 µm (mean 27.49 µm). The starches of *C. zedoaria* and *C. malabarica* showed elliptical shape and granular size of 14-46 µm and 1.6-4.2 µm [18]. *Canna edulis* starch also showed granular shape as starch of *C. angustifolia,* while granule size similar to the starch of *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Elephant foot yam) and *Pachyrrhizus erosus* (Yam bean) [19, 20].

Chemical modification of starches and their characterization

The acetyl, hydroxypropyl, carboxyl groups % and the degree of substitution (DS) for acetylated, hydroxypropylated and caboxymethylated starches of *C. angustifolia* were determined as 6.89%, 0.28%, 0.06%,and 0.04, 1.30, 0.75, respectively.

Solubility and swelling power of native and modified starches

Modification of starches showed changes in solubility behavior. The hydroxypropylated and acetylated starches were insoluble in acetone, tetra-hydrofuran (THF), cold water while carboxymethylated starches was found to be soluble in cold water or in water-miscible solvents. All modified starches showed a significantly higher % light transmittance when compared to unmodified ones. The results also revealed that the % light transmittance of all modified starches was reduced with the duration of storage (24 to 72 h). The effect of acetylation on paste clarity has been widely exported for various types of starch [21, 22]. Figs. 1 and 2 depict the changes in swelling power and solubility % of native and modified starches of *C. angustifolia* with respect to different temperatures (40°C, 50°C 60°C, 70°C, 80°C and 90 °C). The increase in solubility and swelling values was observed in all types of modified starches when temperature increased. Carboxymethylation significantly increases the swelling power and solubility of modified starches of *C. angustifolia*. At 40°C, maximum solubility of 5.46% was observed in carboxymethylated starches, in comparison to acetylated and hydroxypropylated starches while native starch was found to be insoluble.

Table 1. Density, pH, water absorption capacity and gelatinization temperature of native and modified starches

Species	Density (g/ml)	pH	Gelatinization temperature(^{0}C)	Water absorption capacity (g/g)
C.angustifilia (CA)	1.46±0.00	7.30±0.011	97.03±0.00	1.93 ±0.01
AcCA	1.45 ±0.03	4.06±0.02	81.90±0.42	1.86 ±0.04
HPCA	1.42 ±0.06	9.60±0.01	75.20±0.69	2.68 ±0.20
CMCA	1.12 ±0.09	3.65±0.03	68.70±0.49	3.48±0.03

Values are the mean of three replicates±standard deviation
Ac-acetylated, HP- hydroxypropylated, CM-carboxymethyl, CA-*C. angustifolia*

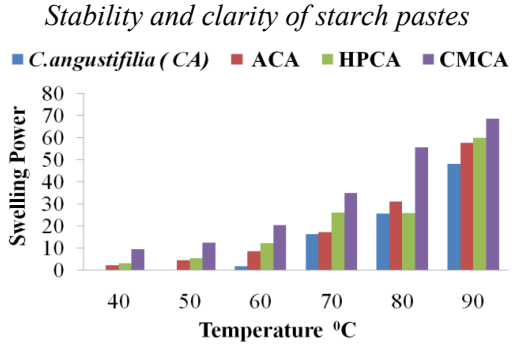


Fig. 1. Swelling power of *C.angustifolia* native and modified starches

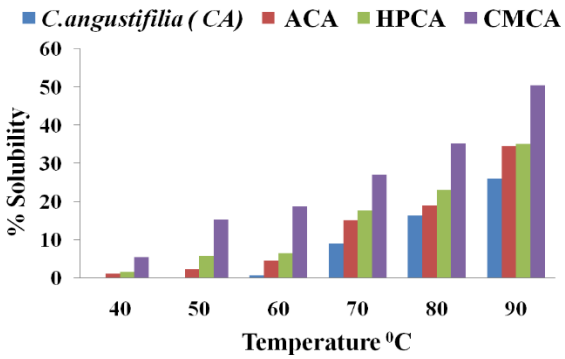


Fig. 2. *C. angustifolia* native and modified starches solubility % at different temperatures

Results of starch paste clarity/% transmittance (T) behavior of native and modified starch are depicted in Fig. 3. Modification improved the paste clarity of the starches as shown by an increase in % light transmittance. All modified starches showed a significantly higher % light transmittance when compared to the native starch. The % light transmittance of modified starches were found to be reduced with duration of storage. Maximum paste clarity (9.889% T) was observed in *C. angustifolia* native starch (Fig. 3) which was reduced to 1.578 % T after 72 h.

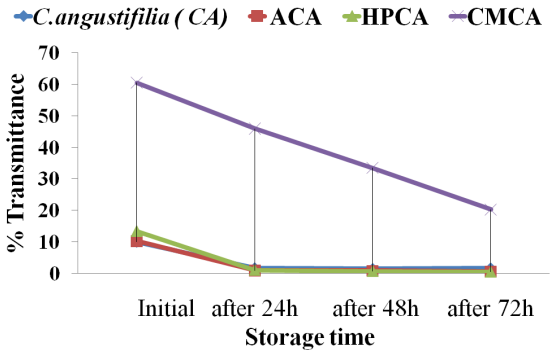


Fig. 3. Impact of storage time on light transmittance (%) of native and modified *C. angustifolia* starch pastes

Water absorption capacity (WAC)

All modified starches showed significantly higher values in comparison to native starch. Results for water absorption capacity (Table 1) of native and modified starches showed significant variation in binding ability of different starch. Similar behavior was observed in starches of other species, this difference in WAC of different species may be due to the difference in amylose/amylopectin percentage and difference in chain length distribution [23]. Introduction of hydroxypropyl group through hydroxypropylation increases the WAC of modified starch which considerably enhances applicability for different industrial use. It is reported that WAC of starch increased with increase in temperature with the addition of more hydrophilic groups [24]. This property of a particular starch may be potentially useful for different industrial purposes.

Modification of starch showed an increase in solubility and swelling properties. The increase in swelling power and solubility for an acetylated starch of *C. angustifolia was* consistent with observations made by other researchers [21, 22, 25]. This tendency in the present study is in reasonably good agreement with the findings in previous studies with acetylated and hydroxylpropylated potato starches [26, 27]. It is observed that CMS granules readily swelled, even at 40°C, compared with f native starch of *C. angustifolia*.

Development of biofilms and their properties

The thickness of films varied from 0.040 to 0.160 mm. Uniform, transparent, translucent, and flexible films were obtained and they were easily removed after drying. The upper side of the film was observed duller while other side was shiny (Fig.4). The acetylated and hydroxypropylated starches showed good characteristics to form films, they were more transparent and easily removed from the cast plate while carboxymethylated starch blends were not found suitable for film preparation. The dried bentonite- and lignin-blended films appeared pale yellow in color. No pores or cracks were observed in films prepared with lignin and bentonite with *C. angustifolia* starches (native and modified).

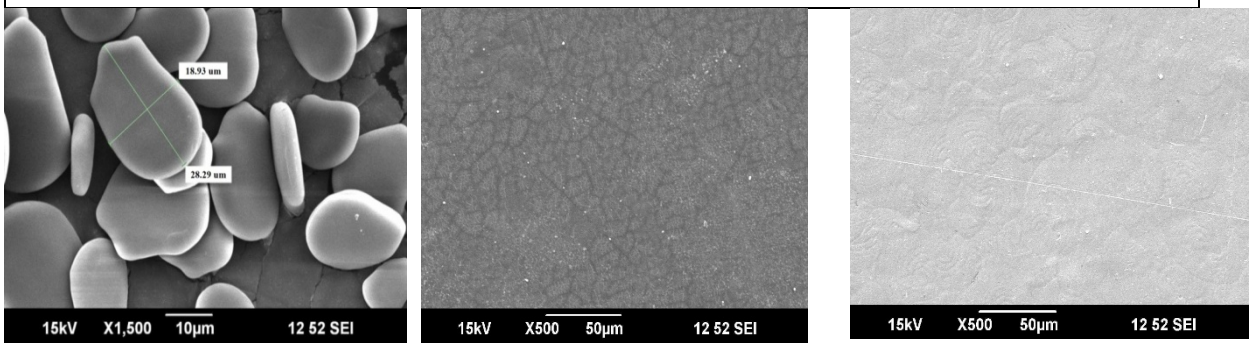
Film opacity and swelling %

Opacity is an important characteristic of the film. Transparent films showed low absorption values. Table 2 indicates the opacity results of different blends. Phytopolymer-starch has received considerable attention because of its biodegradable nature and abundant availability, as well as low cost [28-30]. The acetylated and hydroxypropylated starches were more transparent, having good characteristics and were easily removed from the plate. Filmogenic suspension containing carboxymethylated starches was difficult to handle in casting technique of film preparation. Blending polysaccharides with other polymers, *i.e*. chitosan and polyvinyl alcohol, improved mechanical properties and applications of starches. The surface morphology of different blends was found to different and showed different transparency and opacity. *Curcuma* starches, polyvinyl alcohol and chitosan were found to be compatible. Starch-based films incorporated with lignin, chitosan, bentonite were compact, flexible and translucent in comparison to native starch-based film. The results revealed that use of other polymers with starch could be beneficial for cost reduction and also improve properties in comparison to native and modified starches of species. The chemical structure and physical characteristics of the polymers were changed due to blending. Polymer blending offers wide possibilities of preparing cheap biodegradable materials with potential mechanical properties. SEMs studies revealed the compatibility with different additives.

Fig. 4. Polymeric film

Table 2. Physico-chemical characteristics of native and modified starch films

Film composition	Swelling %			Film opacity [AU nm]	Tensile strength (kgf/cm^2)
	Water	Hexane	Oil		
Ac.CA+P	0.05	Resistive	0.00	100	427.5
HPCA +P	2.41	Resistive	0.42	35.48	310.29
CA+B+P	0.56	Resistive	0.00	0.00	159.41
Ac.CA+B+P	0.45	Resistive	0.04	0.00	143.32
HPCA+B+P	0.00	Resistive	0.01	0.00	177.89
CA+CHI+P+Ac	0.24	Resistive	0.00	100	202.69
AcCA+PVOH	0.00	Resistive	0.03	100	391.79
CA+CHI+B+P	0.04	Resistive	0.24	0.00	127.92
CA- *C. angustifolia*, Ac- acetylated, B-bentonite, P-plasticizer, HP-hydroxypropylated, CHI-chitosan, PVOH-polyvinyl alcohol					



(a) **(b)** **(c)**

Fig. 5. SEM of granules of *C. angustifolia* (a), modified *C. angustifolia* starch blended film (b) and starch and chitosan blended film (c).

Mechanical analysis of starch-based polyfilms

The native and modified starches blended films tensile strength is depicted in Table 2. Results revealed that the starch modification and different additives showed differences in mechanical properties-tensile strength of polysaccharide films. The films thickness varied between 0.040-0.160 mm. The acetylated *C. angustifolia* blend showed maximum tensile strength (427.5kgf/cm^2), followed by modified *C. angustifolia* and PVOH blend (391.79 kgf/cm^2).

Microscopic studies

The SEM of modified *C. angustifolia*: plasticizer blend, and polyvinyl alcohol:mucilage blends has shown particulate morphology. SEM of the surfaces of the acetylated *C. angustifolia* and plasticizer blend is shown in Fig.6b. The appearance of film is smooth, homogeneous, exhibit characteristic patterns on the film surface. These patterns represent the withered ghost granules of starch. The surface of starch and chitosan blend film (Fig. 6c) showed a continuous matrix without cracks with good structural integrity. It was flat and compact with very sparsely distributed small particles without any phase separation.

CONCLUSIONS

Non-degradable and carcinogenic nature of synthetic polymers are posing great threat to environment and human health. This study shows that the use of polysaccharides from non-conventional sources for the development of environmentally friendly polymer products has broad applicability. This study confides on the isolation and modification of *C. angustifolia* starch into acetylated, hydroxypropylated and carboxy methylated products. The starch content of *Curcuma angustifolia* was estimated as 38.11%. Scanning electron microscopy of starch showed round, oval to elliptical, oblong, triangular and asymmetrical shapes, while mean granule length and width were observed 34.62 µm and27.49 µm, respectively.

The acetyl, hydroxypropyl, carboxyl group % and degree of substitution (DS) for acetylated, hydroxypropylated and caboxymethylated starches of *C. angustifolia* were determined to be 6.89% , 0.28%, 0.06%, and 0.04, 1.30,0.75, respectively. The introduction of acetyl, hydroxypropyl and carboxymethyl groups severely affects the properties of native starches. It reveals that this species may be the potential source of starches for

industrial use. The good swelling power makes it a promising pharmaceutical incipient. The modification of starch with the introduction of hydrophobic and hydrophilic groups improves the properties of starch and biofilms, and shows that *C. angustifolia* starches hold great potential for commercial utilization.

The films thickness varied betwen 0.040-0.160 mm. The acetylated *C. angustifolia* blend showed maximum tensile strength (427.5 kgf/cm^2), followed by modified *C. angustifolia* and PVOH blend (391.79 kgf/cm^2). The results indicate that composition of native and modified starch of *C angustifolia* with other synthetic and natural polymers improves the strength of biofilms and thus may be useful for different purposes. The use of underutilized phytopolymer sources offers tremendous possibilities of preparing inexpensive ecofriendly materials with potential mechanical and thermal properties. The applicability of *C. angustifolia* starch polymer may be further explored in the preparation of super-absorbent hydrogels, flocculants, adhesives, and pharmaceuticals for drug release, etc.

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