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ISOLATION OF STARCH FROM *CURCUMA LONGA* L. AND ITS CHARACTERIZATION

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Turmeric starch, Gel permeation chromatography, Viscosity, *Curcuma longa*

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ABSTRACT: The present work was aimed to evaluate the different properties of turmeric starch. Starch has been an endless subject of research for many years. It is an inexpensive, readily available compounds with considerable applications in the food as well as non-food. Turmeric starch was isolated from fresh rhizomes of *Curcuma longa* L. and *Curcuma Caesia* (Zingiberaceae). In this article, we established an easy and simple method of isolation of starch from the fresh rhizomes of turmeric species. Along with physical, physiochemical characteristics were studied like, microscopical analysis size and shape, particle size distribution (2-32 µm), molecular weight determination by gel permeation chromatography (GPC) (372267g/mol), pH of turmeric starch solution (5.6), specific surface area by BET analysis (0.69 m^2/gm), moisture content (15%), true density (0.568 g/cm^3), Iron content (less than 10 ppm), a viscosity of starch solution (1209 cP), and gelatinization temperature (80 °C), *etc*. Remarkably turmeric starch has shown less consistency than other starches and temperature-dependent gelatinization property within 20 min at 80 °C. The main aim of this article is to increase the utility of starches in industries.

INTRODUCTION: Starch is a carbohydrate, and it is a good source for the energy of the human diet. It consists of amylopectin (70-80%) and amylose (30-20%) units [1] by glycosidic linkage. Generally, starch is isolated from potato, maize, wheat, tapioca and rice. Turmeric and ginger are also found to be a good source of starch, which has been used in the food and pharmaceutical industry. Starch is the main form of carbohydrate storage found in green plants. Starch is a polymer it contains the number of glucose (glucopyranose) units with linear alpha (α) 1, 4 linkages (amylose), and alpha (α) branched 1, 4 and 1, 6 linkages (amylopectin).

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Starch is a main source of energy, and it is abundantly present in stem tubers (potatoes), roots (tapioca), cereals (maize), grains (oats, barley, and rice), flour (wheat), dried beans (pinto beans, kidney beans, black-eyed peas, and split peas), seeds (bixin) and rhizomes (turmeric, ginger), etc. In industry, starch has been used as an excipient, diluent, binder, gelling agent, adhesive, water retention, thickener, texture etc. [2]. While in food starch work as a nutritive stabilizer [3]. High-amylose starch produces strong and elongated films [4] and is suitable for making biodegradable plastics, and small granule starch is an appropriate polyethylene film filler.

Various physical, chemical, and enzymatic modifications will change and improve the functional properties of starch and facilitate its utilization for different purposes [5]. Biochemically starch is stored as glycogen and split into its simpler metabolites [6].

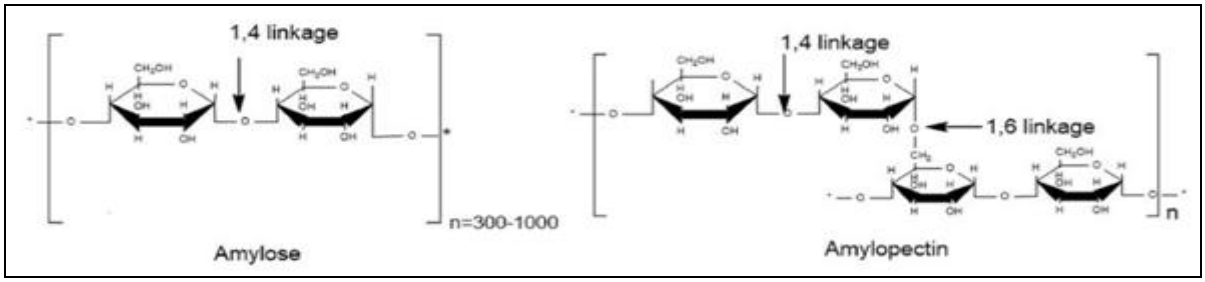

FIG. 1: STRUCTURAL FORMULA OF STARCH POLYMER

India is the biggest producer, consumer, and exporter of turmeric in the worldwide, 80% turmeric produced by India [7]. Other than India's major producers are Thailand, Vietnam, and other Southeast Asian countries, Central Latin America and Taiwan. Various species are cultivated in South East Asia in India. The primary use of turmeric powder was coloring and flavoring agent in food, especially for curries as well as for dyeing. It also has several properties like analgesic, anti-inflammatory, anti-bacterial, anti-cancer, anti-diabetic, *etc*. [8] Also used in traditional functions, and in cosmetics to improve skin nature. Turmeric rhizome contains about 60-70% carbohydrates, 6-13% water, 6-8% protein, 5-10% fat, 3-7% dietary minerals, 3-7% essential oils, 2-7% dietary fibre, and 1-6% curcuminoids [9]. While the major portion of the residue is predominantly starch. Starch has been an endless subject of research for many years. It is an inexpensive, readily available material with considerable applications in the food as well as non-food. Studies on the isolation of starch from turmeric rhizome and its parameters are very limited. Jyothi *et al.* have extracted starch from two species of Curcuma, such as *Curcuma zedoaria* and *Curcuma malabarica*; no major differences have been reported between these two species in respect of granule size, and shape[10].

MATERIALS AND METHODS:

Plant Material: Turmeric rhizomes (*Curcuma longa* sp) and (*Curcuma caesia* L.) grown in Sangli (Maharashtra) were collected and used in this study. The 10-months old non dried rhizomes were harvested for isolating of starch in the laboratory.

Isolation of Starch from *Curcuma longa* Rhizomes: 250 gms of fresh turmeric rhizomes (both Curcuma species) was peeled off, and chopped into small pieces. The chopped pieces were soaked in water for about 12 h. Then remove the soaked material and grind to a fine paste and make it as thin slurry. Pass the slurry through double-layered of muslin cloth. The supernatant layer was decanted and mass allowed to settle down for further about one hour. To get rid of impurities, turmeric starch was washed twice with water in the centrifuge. Fallowed by soxhelation with methanol to remove the color matter from starch. Collected starch was dried in an oven at 400 °C.

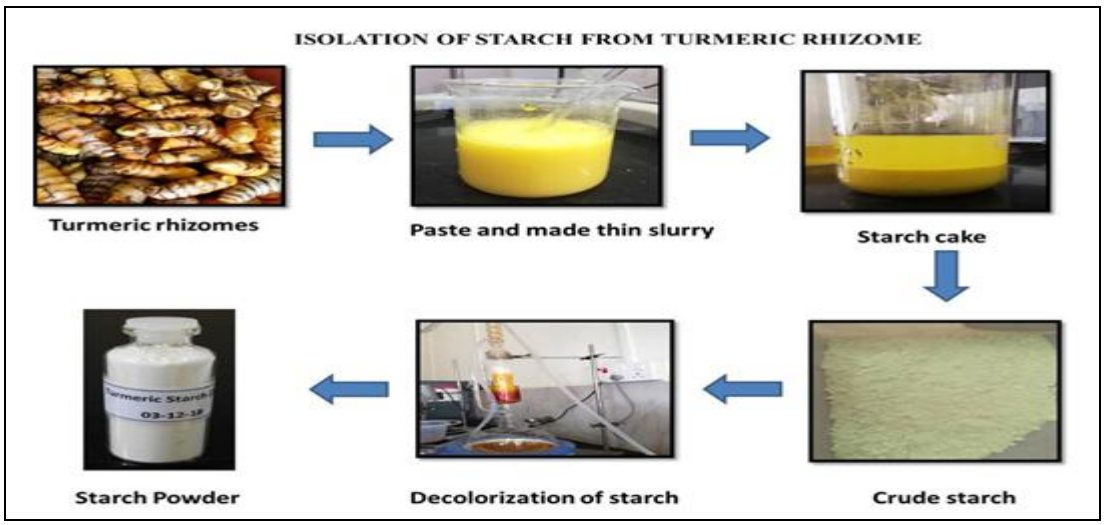

FIG. 2: ISOLATION OF TURMERIC STARCH FROM RHIZOMES PICTORIAL REPRESENTATION

Microscopic Characteristics of Turmeric Starch:

Microscopy Analysis: A small amount of starch was mixed with water and observed under a compound microscope under 10X and 45X. The pictures were shown under results and discussions.

Starch Morphological Analysis by Scanning Electron Microscopy: The microstructure of the isolated starch sample was examined by employing a scanning electron microscope (SEM) previously used method by Dhanalakshmi, Kuttigounder. In the SEM analysis, the starch sample has interacted with some focussed beam of electronic energy, It scanned the sample and visualized its images on display [11].

Swelling Power and Solubility of Starch: The swelling power and percentage solubility of isolated starch were determined by using the Leach method [12]. Take 1 g of sample was dissolved in 30 ml of distilled water in a preweighed centrifuge tube. Centrifuge tubes were kept in a water bath maintained at different temperatures (50, 60, 70, and 80 °C) with continuously stirring for 30 min, leads to the forming of a dispersion. The dispersion was centrifuged at 3000 rpm for 15 min.

The supernatant was completely recovered, and the swollen starch sediment was weighed. The supernatant was then evaporated overnight (110 °C). Swelling power and percentage solubility of starch was determined in triplicates. Swelling power was calculated by using equation (1), solubility was calculated by using equation (2).

Swelling power = Weight of wet sediment (g) / Weight of dry starch (g)….(1)

Percentage solubility = Weight of dry supernatant (g) / Weight of dry starch (g) ×2 ×100….(2)

Water-binding Capacity Determination: The water-binding capacity of isolated starch was determined by Medcalf and Gilles method [13] with some modifications. About 1.0 g of starch was dispersed in 20 ml of distilled water, and the suspension was shaken for 1 h on a shaker. The later suspension was centrifuged for 10 min at 2200 rpm. Then the supernatant was decanted, and the starch deposit was allowed to settle down for 10 min and then weighed. Water binding capacity was calculated by using equation (3).

Water binding capacity (%) = Weight of the residue – the weight of sample ×100 / Weight of sample….(3)

Determination of Moisture Content of Starch by Hot Air Oven Method: The moisture content of isolated starch was determined by hot air oven method [14]. Approximately 5 g of sample was weighed in a pre-weighed evaporating dish. The dish was placed in a preheated and maintained hot air oven at 105 °C for 8 h [15]. The sample was cooled in a desiccator until it reached room temperature and measured the weight. The loss in weight was determined in triplicates and reported as the moisture content on the percent basis.

Gelatinization Temperature of Starch: Gelatinization properties of turmeric starch were determined by the AACC official method [17]. Prepare 10% starch suspension and kept in boiling water bath with continuous stirring. Record the temperature when it gets gelatinized with the help of a thermometer. The viscosity of gelatinized starch was done by Fungi lab viscolead pro Viscometer. The viscosity was expressed in terms of centipoises (cP).

Specific Surface Area: The specific surface area of starch was determined by BET [18] analysis. The specific surface area of starch was expressed in terms of m^2/gm.

Molecular Weight Determination by Gel Permeation Chromatography (GPC): The molecular weight of isolated turmeric starch was determined by gel permeation chromatography (GPC). For this aglient 1260 equipment with PL aqua gel-OH (Guard column), PL aqua gel-OH 40 columns were used. The flow rate was 1ml/min, injected volume of the sample was 100µl, refractive index detector, and different concentrations of dextrose were used as standard. It reveals an average number of molecular weight (Mn), weight average molecular weight (Mw), molar mass distribution (MWD) of starch [19].

TABLE 1: THE FOLLOWING PHYSICOCHEMICAL PARAMETERS OF TURMERIC STARCH WERE EVALUATED

S. no.	Physicochemical Parameters
1	pH [16]
2	Bulk density [22]
3	Tapped density [22]
4	Compressibility index [20]
5	Flowability [20]

Total Ash Content: Total ash in turmeric sample was determined by dry ash method [21]. Ash indicates total inorganic residue remaining after the total incineration of organic matter. The ash content is an indicator of product quality and the nutritional value of food products concerning mineral content. About 2 g of turmeric powder was weighed in a pre-weighed silica crucible. The sample was charred over a Bunsen burner at 550° - 650 °C for 5 h to oxidize all the organic materials. The inorganic residue that remained in the crucible was weighed. The ash content was calculated from the loss in weight and expressed as a percentage of triplicate samples.

Percentage Ash = Weight of total ash × 100 / Weight of sample….(4)

Limits of Iron in Turmeric Starch: The iron content of turmeric starch was determined by the monograph of corn starch [23].

Standard Iron Stock Solution A: This was prepared by dissolving 863.4 mg of ferric ammonium sulfate in water, 10 ml of 2 N H_2SO4 was added and was diluted with water to 100 ml.10 ml of this solution was pipetted into a 1000 ml volumetric flask, 10 ml of 2 N H_2SO4 was added, and finally the volume was made with water. Standard Stock solution B: 1 µg/ml of iron from standard iron stock solution A in the water was taken to prepare Stock solution B. Standard Iron solution: 10 ml of Standard iron stock solution B was transferred to a test tube, 2 ml citric acid was added, followed by the addition of 0.1 ml thioglycolic acid. 10 N NH_4OH was added until the solution was alkaline to litmus. It was then diluted with water to 20 ml. Sample preparation: 1.5 g of starch sample was dissolved in 15 ml of 2 N HCl followed by filtration. 10 ml of this filtrate was transferred to a test tube; 2 ml of citric acid and 0.1 ml of thioglycolic acid were added. This was followed by the addition of 10 N ammonium hydroxide to the solution was alkaline to litmus. Finally, the volume was made with water to 20 ml.

RESULTS AND DISCUSSION:

Microscopy Results: Microscopy reveals information regarding the shape and size of the starch. Turmeric starch granules mostly in ellipsoid, ovoid and elongated irregular in shape and approximate size were 2-32 µm. 45X, 10X, and iodized slides, respectively, picture shown below.

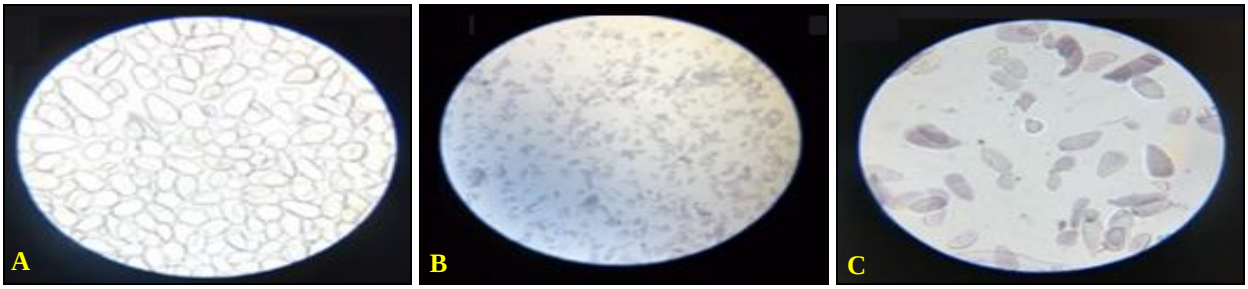

FIG. 3: PICTURES OF NORMAL MICROSCOPY (A) 45X, (B) 10X, AND IODIZED STARCH UNDER 45X (C)

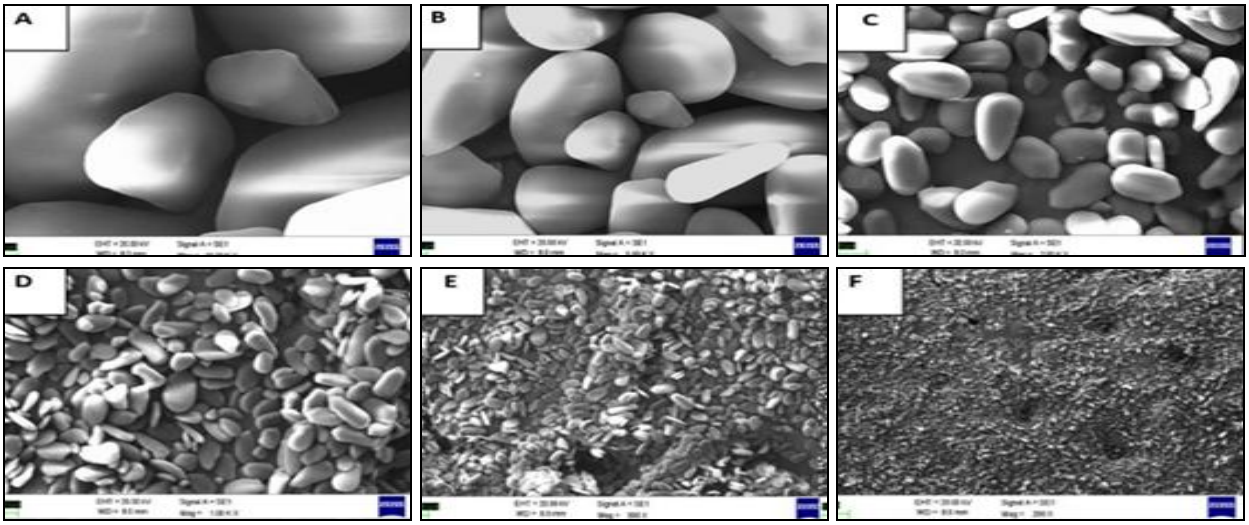

FIG. 4: PICTURES OF SEM ANALYSIS MAGNIFICATIONS: A:10000X, B:5000X, C:2000X, D:1000X, E:500X, AND F:200X

Scanning Electron Microscopy (SEM): Microscopic examinations of the samples were conducted at an accelerating voltage of 20 kV at magnifications of 10000x, 5000x, 2000x, 1000x, 500x, and 200x picture given below.

Swelling Power and Solubility: Both swelling power and percentage solubility properties of turmeric starch were determined. These properties represent evidence of interaction between the amorphous and crystalline areas. Furthermore, it is influenced by amylose and amylopectin characteristics [24]. Greater swelling power shows greater solubility [25]. The results were shown in the below table:

TABLE 2: SWELLING POWER AND PERCENTAGE SOLUBILITY

Temp.	Swelling power (g)	Percentage solubility (%)
50 °C	1.77	0.98
60 °C	2.82	1.78
70 °C	6.02	2.59
80 °C	6.19	4.7

The Molecular Weight of Starch: Molecular weight (Mw) of turmeric starch was determined by gel permeation chromatography (GPC). It was found to be 372267 g/mol.

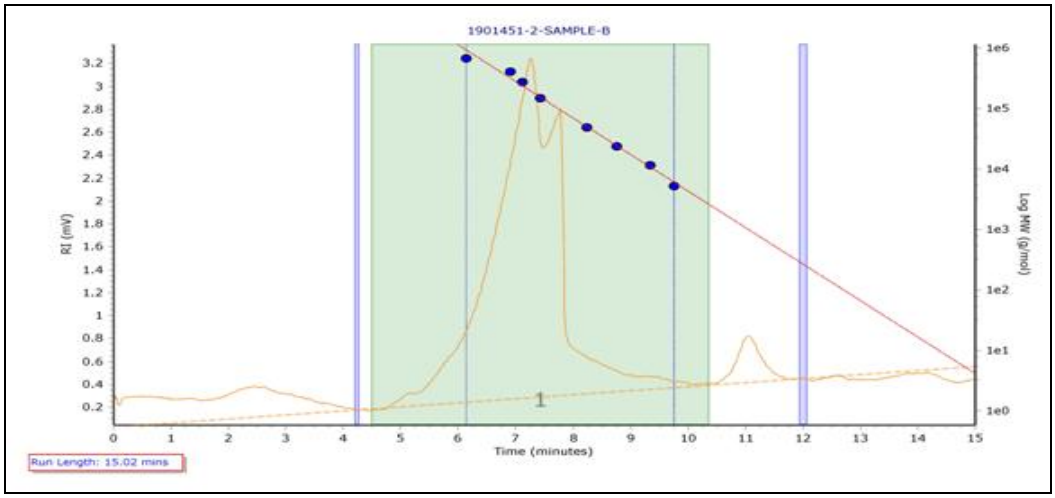

FIG. 5: CHROMATOGRAM OF TURMERIC STARCH

TABLE 3: AVERAGE MOLECULAR WEIGHTS

Peak	Mp(g/mol)	Mn(g/mol)	Mw(g/mol)	Mz(g/mol)	Mz+1(g/mol)	Mv (g/Mol)	PD
Peak 1	193643	108677	372267	1122981	2433723	95737	3.425

Mp: peak molecular weight, Mn: number average molecular weight, Mw: weight average molecular weight, Mz: Z weight average molecular weight, PD: polymer distribution

TABLE 4: PROXIMATE RESULTS OF SOME PARAMETERS

Parameters	Observed values
Water binding capacity (%)	82 ± 0.2
Moisture content (%)	15 ± 0.2
Percentage of ash content (%)	0.65 ± 0.2
pH	5.6 (acidic pH)
Gelatinization temperature (°C)	80 ± 0.2
Hot Viscosity (cP)	1209
RT Viscosity (cP)	5026
Specific surface area (m^2/gm)	0.68
Compressibility index (%)	9.85 (Excellent)
Tapped Density (g/ml)	0.568
Bulk Density (g/ml)	0.512
Iron content (ppm)	Less than 10 (< 10)

The starch was isolated from both *Curcuma longa* and *Curcuma caesia*. In those Curcuma species, *Curcuma longa* (20.8%) contain more percentage of starch than *Curcuma caesia* (14.7%).

So, all given parameters were evaluated with *Curcuma longa* starch because it is practically proved as more than *Curcuma caesia*. All physiochemical and microscopical properties were evaluated for starch. In this study, isolated turmeric starch was very pure; it is clearly understood by microscopy and scanning electron microscopy (SEM) analysis pictures; those were clearer than earlier studies.

CONCLUSION: The rhizomes of *Curcuma longa* and *Curcuma caesia* can be studied as a good unconventional source of starch for many applications. In those, *Curcuma longa* contains more percentage of starch than *Curcuma caesia*. Viscosity properties and rheology flow properties were checked for isolated turmeric starch.

The size (2-32µ) and elongated oval shape (elliptical) of the turmeric starch grains were detected with microscopy and scanning electron microscopy (SEM). The molecular weight of starch was determined by gel permeation chromatography.

The specific surface area of turmeric starch was determined by BET analysis. These starch granules may be used as a matrix for encapsulation techniques. Turmeric starch is a common food ingredient, used in thickening of soups, and in making of syrups along with other sugars. It is easily modified, and finds many uses in industries as adhesives, in paper products, as an anti-sticking agent, and textile manufacturing.

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