Extraction and scanning electron microscopic studies of
*Curcuma angustifolia* Roxb. starch

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Starch is the main carbohydrate produced by all green plants and is basically found in the seeds, fruits, tubers, roots and stems of various plants, notably corn, potatoes, wheat and rice. Starch varies widely according to its source owing to its granular characteristics. About 50% of starch produced is used in food industry, either as food additive or to improve functional properties and shelf life of food products. *Curcuma angustifolia* Roxb., commonly known as *Tikhur* in Hindi, occurs wildly in many parts of India. It is traditionally recognized as medicinal plant and also contains starch in its rhizome. In some forest tubers extraction of starch is simple, whereas this is not always so with other tuber starches. Maximum recovery of starch with economical extraction is thus important. Therefore, extraction of the starch in *Tikhur* was done using 1% ammonium oxalate and 0.03 M ammonia solution and purification of starch was done according to the method of Badenhuizen, 1964. The yield of starch in 0.03 M ammonia solution and 1% ammonium oxalate was obtained 38.46 and 37.64%, respectively. The granular shape and size of starch granules were recorded using Scanning Electron Microscope. The shape of *C. angustifolia* starch granules were small rounded, oval to elliptical, spherical, elongated and 3.32 µ to 32.55 µ in length and 2.29 µ to 8.47 µ in width. The study demonstrated that for extraction of starch from *C. angustifolia* 0.03 M ammonia solution method described is best and yields significant quantity of starch. The results would aid the authentication and to check the adulteration of starch of this species.

**Keywords:** Rhizome, Starch, Starch granules, *Curcuma angustifolia*, Tikhur, Food product.

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**Introduction**

Tropical tubers contain starch as the major component and are staple or subsidiary food for the low income group. In Asian countries like India, China, Thailand, Indonesia except *Manihot esculenta* Crantz (Cassava) and *Ipomoea batatas* Lam. (Sweet potato), other tuber crops have not been exploited for extraction of starches. Studies on different starches in India and elsewhere have revealed wide diversity in starch characteristics of tuber crops and the possibility of using these native starches instead of chemically modified starches.

In some forest tubers like cassava extraction of starch is simple; the isolated starch is white in colour and relatively free from other chemical impurities, whereas this is not always so with other tuber starches. The settling of starch granules is often hindered by presence of various components like proteins, fats, mucilage’s and latex, leading to lowering of quantity of extracted starch. The long time for extraction may promote microbial growth and also deteriorate colour and quality of starch. Economical extraction and maximum recovery of starch from forest tubers is thus utmost important. Hence different chemicals were used for extraction and improving yield of starch from tropical tubers.

Starch occurs in plants as granules having a characteristic size and shape for each plant species. The suitability of starch for a specific use depends on size and shape of the starch granules. In *Curcuma malabarica*, *C. caesia* Roxb., *Curcuma longa* Linn. syn. *C. brog* Valeton, *C. amada* Roxb. and other *Curcuma* species except *C. angustifolia* Roxb. starch yield have been estimated and granular shape and size of the starch granules have been studied. Suitability of *Curcuma angustifolia* starch for pharmaceutical excipient has also been reported.

*Curcuma angustifolia* Roxb., commonly known as *Tikhur*, occurs in the hilly tracts of Central India, West Bengal, erstwhile Bombay and Madras provenances and some of the lower Himalayan ranges. The plant grows wild in the moist and cool areas at altitudes of about 2000 m and is cultivated to...
INDIAN J NAT PROD RESOUR, SEPTEMBER 2012

408

a small extent. The plant is known to yield volatile oil from leaves and possess antimicrobial properties\textsuperscript{10,11}. The rhizomes of \textit{C. angustifolia} contain starch and is reported to be used by tribals of India for the preparation of milk puddings. Since, starch of \textit{Tikhrur} has received little attention, despite their potential application in food and textile industries. The present investigation discusses the yield determination \textit{vis-a-vis} scanning electron microscopic studies of starch granules of this hitherto unexploited starch source.

**Materials and Methods**

The rhizomes of \textit{C. angustifolia} were collected from Non Wood Forest Produce Garden, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India (Plate 1).

**Extraction of starch**

After washing and peeling of the thin skin, rhizomes were cut into small pieces and subsequently passed through the extraction procedures by two different methods. In first method the cut rhizomes pieces of 0.1 cm were dipped overnight in 0.1% solution of sodium bisulphite\textsuperscript{12}. The samples were then homogenized in a laboratory mixer and extraction of the starch was done in 1% ammonium oxalate\textsuperscript{5}. The suspension was then filtered through muslin cloth and kept for sedimentation of starch. The residue retained on muslin cloth was again homogenized in a laboratory mixer and the extraction of starch was done in ammonium oxalate. The process was repeated 3-4 times or till all the starch was extracted from the material.

In second method the sample was homogenized directly in a laboratory mixer and extraction of starch was done in 0.03 M ammonia\textsuperscript{6}. The filtrate so obtained was collected separately and kept for sedimentation. The residue retained on muslin cloth was again homogenized in a laboratory mixer and the extraction of starch was done in 0.03 M ammonia. The process was repeated 3-4 times till all the starch was extracted from the material. Afterward the supernatant layer was decanted from the sediment starch layer and the sediment was collected.

The sediment starch of both procedures was collected separately and purified\textsuperscript{5}. The sediment was then treated with saline suspension at room temperature, followed by shaking the saline suspension with toluene (0.1 volume) to denature and to ensure removal of residual cytoplasmic proteins without causing degradation of starch. The sedimentation cycle was repeated 2-3 times. After sedimentation of the starch granules, the protein-toluene layer was discarded and the starch was defatted by refluxing with methanol (80%), washed with acetone and ether, and dried under reduced pressure. The yield of starch was then quantified gravimetrically.

**Scanning Electron Microscopy**

Starch sample was taken in a small stoppered glass tube and dehydration of starch was done serially in 30, 50, 70 and 90% ethyl alcohol for few minutes. Afterwards absolute alcohol was added and samples were kept for few hours. The solvent was then decanted and absolute alcohol and amyl acetate (1:1) ratio was added and kept for 24 hours. After decanting the alcohol and amyl acetate mixture the starch sample was preserved in amyl acetate until used for gold coating.

For preparation of sample for gold coating amyl acetate was decanted from the glass tube and starch was kept for air drying for few minutes. Afterwards gold coating tape was pasted on the circular stub and starch granules were sprinkled on the stub. The prepared sample stub was coated with gold for 30-45 seconds by using gold coating machine. The stub was then removed from gold coating machine. The stub was then viewed in Stereoscan Leica 410 microscope at low magnification 500 and EHT 5 KV using SE1 detector. The size of starch granules were measured by point to point method and photographed through camera attached with the SEM.

**Results and Discussion**

The yield of \textit{C. angustifolia} starch in 0.03 M ammonia solution was 38.46% whereas it was 37.64% in 1% ammonium oxalate solution after removal of

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Plate 1—Rhizome of \textit{Curcuma angustifolia}
the impurities. The extraction of starch in both the extraction medium resulted almost similar quantity of starch. In both the procedures feasible quantity of native starch was obtained. Repeated washing of *C. angustifolia* starch with saline solution and then with saline solution with toluene yielded low protein starch. In different forest species, viz. *Aesculus assamica* Griff., *Amorphophallus campanulatus* Blume ex Decne, *Canna edulis* Ker-Gawl., *Careya arborea* Roxb., *Casimiroa edulis* Llave & Lex., *Shorea robusta* Gaertn.f., *Stephania glabra* Miers., *Pueraria thomsonii* Benth., *P. tuberosa* DC. and *Quercus leucotrichophora* K.C. Velayudhan, V.A. Amalraj & V.K. Muralidharan, *C. caesia* Roxb., *C. longa* Linn., *C. amada* Roxb., *C. aromatica* Salish., *C. zedoaria* Rosc., *C. aeruginosa* Roxb., *C. haritha* J.K. Mangaly & M. Sabu, *C. raktakanta* J.K. Mangaly & M. Sabu and *C. sylvatica* Valeton were reported to be 21.40, 19.86, 18.00, 10.22, 15.00, 14.06, 14.10, 14.32, 14.20 and 10.34%, respectively. The starch yield in *C. malabarica* K.C. Velayudhan, V.A. Amalraj & V.K. Muralidharan was reported as 12.10, 34.03, 60.3, 50.30, 26.30, 15.60, 82% (dry weight basis), respectively was reported as 45, 56-58 and 82% (dry weight basis), respectively was reported. The 0.03 M ammonia solution was used for extraction of starch from tropical forest tubers, viz. *Manihot esculenta* Crantz (Cassava), *Dioscorea alata* Linn., *D. esculenta* Burkill, *D. rotundata* Poir., *Ipomoea batatas* Lam. (sweet potato) and *Xanthosoma sagittifolius* (Linn.) Schott. Ammonia acts by complexing with the mucilaginous material releasing the starch granules. Thus use of ammonium solution enables faster settling of starch in less viscous slurry. The short residence time also prevents microbiological damage of the starch. Ammonia was found most effective in improving the yield of starch in aroids and yams.

The Scanning Electron Microscopy (SEM) studies revealed that the size and shape of starch granules of *C. angustifolia* were small, rounded, oval to elliptical, spherical, elongated 3.32 ±32.55 µm in length and 2.29 ±8.47 µm in width (Plate 2). The size of the granules observed in the present investigation is coherent with the average size of starch granules ranging between 3-20 µm for small granules and 20-48 µm for large granules. The elliptical shape and granular size of 14-46 µm and 1.6-4.2 µm, respectively of starch granules were reported in *C. zedoaria* and *C. malabarica*. The SEM studies revealed the variation in shape of granules, with round, elliptic, irregular and polygonal. Similar studies have also been reported in *C. amada*. The shape of starch granules of *Stephania glabra* and *Pueraria thomsonii* were reported spherical and polygonal. In *C. zedoaria*, *C. raktakanta*, *C. caesia*, *C. aeruginosa* and *C. aromatica* granular size 6-25 µm while in *C. malabarica*, *C. longa* and *C. sylvatica* granular size 10-40 µm were reported. The starch granules size of *Casimiroa edulis*, *Careya arborea*, *Aesculus assamica*, *Canna edulis*, *Pueraria tuberosa* and *Pachyrhizus erosus* (Linn.) Urban were reported in the range of 3.6-21.6 µm, 7-24 µm, 5-40 µm, 13.05-57.63 µm and 1.6-22.8 µm and 6-35 µm, respectively. Granular shape of starch of *C. angustifolia* resembles to the starch of *Canna edulis* and granule size resembles to the starch of *Amorphophallus paonifolius* (Dennst.) Nicolson (Elephant foot yam) and *Pachyrhizus erosus* (Yam bean). In *Pachyrhizus ahipa* geometric form starch granule with size of 5-35 µm was reported.

**Conclusion**

The study demonstrated that for extraction of starch from *C. angustifolia* 0.03 M ammonia solution method described is best yielding significant quantity and better quality of starch. The findings can be used to check adulteration and to provide authentic sample of starch of *Tikhur*. From the results, it can be concluded that *Tikhur* could become source of starch for commercial utilization.

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