Chapter 2

To extract and characterize starch of the kernel of *Euryale ferox* seed

2.1 Introduction

Euryale ferox is cultivated primarily for its edible seeds in India, China, and other Southeast Asian countries. *Euryale ferox* may be harvested at different level of maturity *viz*., young, ripe, and overripe. Overripe fruits explode, floats on water for few days, later settle at the bottom of the pond. Skilled farmers expertise in harvesting of foxnut used to dive underwater to collect seeds settled at the bottom of the pond while seeds that float on water may be collected by unskilled farmers [39]. According to previous studies, the major edible part of *Euryale ferox* seed is its starchy kernel which constitutes about 77% but remains underutilized in food processing industries [21]. Taking advantage of its compositional benefit that is being rich in starch, it may be possible to convert into a variety of foods to meet the demand of increasing population.

Euryale ferox kernel starch has yet to be harnessed, despite its high starch content. Due to the lack of awareness of this crop among researchers, there has been relatively little work published on the isolation and characterization of *Euryale ferox* kernel starch. *Euryale ferox* is widely produced in Eastern and Northeastern Regions of India particularly in Assam and Manipur. However, there is no information in the literature about the physicochemical characterization and functional properties of the starches isolated from the of *E. ferox* cultivars of Northeast India. It is anticipated that starch obtained from different cultivars may exhibit different physicochemical, functional, and nutritional properties. In the view of the above hypothesis, the purpose of the current study was to isolate and characterize starch obtained from the kernel of *Euryale ferox* seed collected from Assam and Manipur in Northeast India, so as to provide useful facts and figures to suggest potential applications of this unconventional starch.

Furthermore, in recent years, non-conventional and underutilized starch sources with desired physicochemical and functional features that do not require genetic or chemical modification are being investigated for their possible usage in order to meet the growing demand for starch [35]. Therefore, before approving a novel source of starch for industrial application, a significant amount of effort must be done in order to understand its structural, physicochemical, and functional qualities so as to suggest the most appropriate applications in food and non-food industries [37]. The present investigation will be

immensely helpful in the popularization of this underutilized crop and support the local growers as well.

2.2 Materials and methods

2.2.1 Sample collection and preparation

Euryale ferox samples were harvested at the matured stage from the wild habitat ponds of Imphal East (Kangla Siphai village), Manipur Region, India. Another set of *Euryale ferox* samples were collected from the Assam Agricultural University, Jorhat, Assam Region, India. The arils were removed by manual abrasion. Seeds were dried in a tray drier (Model-TD-C, BIOCOCTION, India) at 60^{o}C for 24h up to 10% moisture contents. Then, the dried seeds were stored in a desiccator. The hard shells were removed by beating with a wooden rod, and the kernels were separated and used for starch extraction. The chemicals and reagents used in the experiments were all analytical grade and of high purity analytical grade.

2.2.2 Determination of starch content and isolation of starch from *Euryale ferox* kernel

The starch content of *Euryale ferox* kernels was estimated following the method of Gao et al. [16]. With minor changes, EFKS was extracted using the method of Ezekiel *et al.* [13]. Deshelled kernels (5g) of *Euryale ferox* were steeped in 30mL of distilled water for 24 h at 30°C to soften the kernels. On the following day, the softened kernels were crushed thoroughly in a mechanical blender to get thin slurry. The slurry was clarified using fourfold muslin cloth and the remnants on the filtration cloth were flushed and squeezed. The washing and squeezing process was repeated to extract maximum starch. The purified slurry thus obtained was collected in a beaker and held overnight and the solid materials present in the suspension settled, and the clear liquid was discarded. The solid portion thus remained was washed repeatedly (5 times) with distilled water until the washing water became transparent. The suspension was centrifuged at 700 rpm, the supernatant was thrown out, and the white central portion was gently distributed on petri plates and dried inside a hot-air oven until brittle at 40°C. For further analyses, the dried material was crushed into fine particles and stored in a tightly sealed sample holder at room temperature (30°C).

2.2.3 Chemical composition and amylose content

The moisture, crude protein, crude fiber, crude fat, and total ash content of EFKS were estimated using AOAC (2005) [3]. The amylose percentage of the starch sample was quantified using the iodine-binding technique [34]. Pure potato amylose purchased from Sigma-Aldrich, was used to plot a standard amylose curve. The absorbance at 620 nm was used to quantify the amylose content of the sample.

2.2.4 Morphology of *Euryale ferox* kernel starch

A scanning electron microscope (JEOL JSM 6390 LV, Singapore) was used to examine the granule morphology of EFKS. Double-sided tape was used to spread starch grains finely on the aluminium specimen holder. Platinum coated samples were scanned at a magnification of 5500 times with 15 kV accelerating voltage.

2.2.5 X-ray diffraction (XRD) pattern and relative crystallinity

The X-ray diffractometer (Miniflex, Japan) was used to perform the X-ray diffraction investigation that resulted in the XRD pattern. The sample was exposed to an X-ray beam with a 30 kV acceleration potential and a 15-mA current for the study. Data were generated with a diffraction angle (2θ) ranging from 5 to 50 degrees and a step angle of 0.05 degrees. The percent crystallinity was evaluated by calculating the percentage ratio of area under diffraction peak to total diffraction area as described by Deka & Sit [10].

$$\% \text{ Crystalinity} = \frac{\text{Area under peak} \times 100}{\text{Total area}} \quad (1)$$

2.2.6 Fourier transform infrared (FT-IR) spectroscopy analysis

Fourier transform infrared (FT-IR) (Spectrum 100, Perkin Elmer, SA) spectra were used for determining functional groups present in the sample. Samples were blended with completely dried pure KBr and the blend was pressed into pellets for FT-IR analysis with a resolution of $4cm^{-1}$ in the frequency range of 4000-400 cm^{-1}.

2.2.7 Gelatinization properties

Gelatinization properties of *Euryale ferox* kernel starch was recorded by using differential scanning calorimetry (DSC) (Polyma, NETZSCH Corporation, Germany). Starch (5mg) was blended with 15µL of distilled water, hermetically capped in aluminum pans and left at 4℃ for 12h. Samples were scanned against an empty pan at a heating rate of 10℃/min from 25 to 130℃. The onset temperature (To), peak temperature (Tp), final temperature (Tc), and change in enthalpy during gelatinization (ΔH) were described as the key parameters of the DSC profile.

2.2.8 Thermogravimetric analysis

The thermal properties of starch were determined using TGA instrument (Shimadzu TGA-50). Sample (5 mg) was used, and the temperature was raised from 25 to 400℃ at a heating rate of 10℃/min.

2.2.9 Swelling power and Solubility index

The swelling power and solubility of the complexes were evaluated [22]. Briefly, samples of 100mg (W_1) were blended with 10mL of water. The slurry mixture was vortex mixing for 2 min and then incubated in a hot water bath at 90°C for half an hour. Then, the tubes have been brought to room conditions, and the suspensions were centrifuged at 2000*g* for 30 min and the weight of the sediment was recorded (W_2) while the supernatants thus obtained was carefully collected into a pre-weighed aluminum can (W_3), and kept in a hot air oven at 105°C until the weight remain constant (W_4) and their difference was the weight of the supernatant (W_5). The following formulae were used to determine the solubility index and swelling power.

$$\text{Swelling power (g/g)} = \frac{W_2}{W_1 - W_5} \tag{2}$$

$$Solubility\ (\%) = \frac{W_5}{W_1} \times 100 \tag{3}$$

2.2.10 Pasting properties

The pasting characteristics of *Euryale ferox* kernel starch were assessed using a rapid visco analyzer (Starch Master 2 of Newport Scientific, Australia) by following 13 min of heating and cooling cycle [11]. EFKS (3 g) was blended with 25 mL of distilled water. Samples

were warmed up from 50 °C to 95 °C within 5 min, hold on at 95°C for 2 min, allowed to cool down to 50 °C within 4 min, and finally maintain at 50 °C for 2 min. The pasting parameters such as pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), and final viscosity (FV) were all obtained from the graph and recorded. The obtained viscosity was expressed as cP (centipoises).

2.2.11 Color properties

The color characteristics of EFKS were analyzed using a colorimeter (Ultrascan VIS, Hunterlab, USA). Results were reported in terms of Lightness (L*) values extend from 0 (black) to 100 (white). Positive a* represents redness and negative a* represents greenness while positive and negative b* represents yellowness and blueness respectively.

2.2.12 *In vitro* starch digestibility (IVSD)

The *in vitro* starch digestibility of EFKS in its native, and retrograded form (gelatinized starch which were re-crystalized at 4℃ for 36h) was assessed by employing the procedure of Englyst *et al.* [12]. Briefly, porcine pancreatin (12 g) was suspended in 80mL distilled water, agitated for 10 min, then centrifuged at 3000*g* for 15 min and 54mL of the supernatant was collected. Amyloglucosidase (3.15mL) was diluted in 3.85 mL deionized water and 6mL was collected to combine with 54mL supernatant. In a conical flask, starch (1g) was combined with 20 mL of 0.1 M acetate buffer having pH 5.2, boiled for 30 min with continuous stirring in a hot water bath, and then brought to the normal temperature (37 °C). The enzyme solution (5mL) was then added and incubated in a water bath at 37°C. The hydrolysate (0.5mL) was withdrawn after 20, 40, 60, 120, and 180 min of digestion and combined with 20 mL of 66% ethanol. The hydrolysates were centrifuged for 10min at 3000*g*, and the hydrolyzed glucose contained in the supernatant was determined using the GOPOD reagent (GAGO-20 assay kit).

Based on the hydrolysis rate, starch components were categorized as RDS, SDS, and RS using the formulae below:

$$\text{RDS}\ (\%) = \frac{G_{20} - G_0 \times 0.9}{TS} \times 100 \quad (4)$$

$$\text{SDS}\ (\%) = \frac{(G_{120} - G_{20}) \times 0.9}{TS} \times 100 \quad (5)$$

$$\text{RS}\ (\%) = \frac{\text{TS} - (G_{120}) \times 0.9}{\text{TS}} \times 100 \quad (6)$$

where, G_{20} and G_{120} are glucose released after 20 and 120 min of hydrolysis, respectively. TS is the total starch contents in the sample.

2.2.13 Statistical analysis

All analyses results reported are mean of three replications and data were expressed as mean ±SD. One-way ANOVA was used in the statistical analysis, and statistical significance was assessed at $p < 0.05$ by Tukey LSD using SPSS 25.0 software (IBM Corporation, Chicago, IL, USA) (Least Significant Difference).

2.3 Results and Discussion

2.3.1 Specimen identification

Euryale ferox specimens have been taxonomically identified in the Department of Botany, Gauhati University, Assam (Accession No. GUBH 19000).

Fig. 2.1 Herbarium of *Euryale ferox*

2.3.2 Starch content, starch yield, chemical composition and amylose contents

The starch contents of the *Euryale ferox* kernels from Assam and Manipur cultivars was 68% and 71% respectively, this is in agreement with the values reported by Zhao *et al*. [39]. The yields obtained from *Euryale ferox* kernel starch-Assam (EFKS-A) and *Euryale ferox* kernel starch-Manipur (EFKS-M) were 36.13 and 40.53 %, respectively. Starch content of *Euryale ferox* was lesser than rice (82.7%) but remarkably higher than a tuber

rhizome (49.8%) [24]. The low yield of starch from *E. ferox* can be attributed to the difficulty in starch isolation owing to the presence of bran layer. Starch recovery from the kernels of *Euryale ferox* may be enhanced by removing the bran layer before steeping in water for starch isolation so that the color pigment from bran layer does not contaminate the white starchy portion while grinding and steeping.

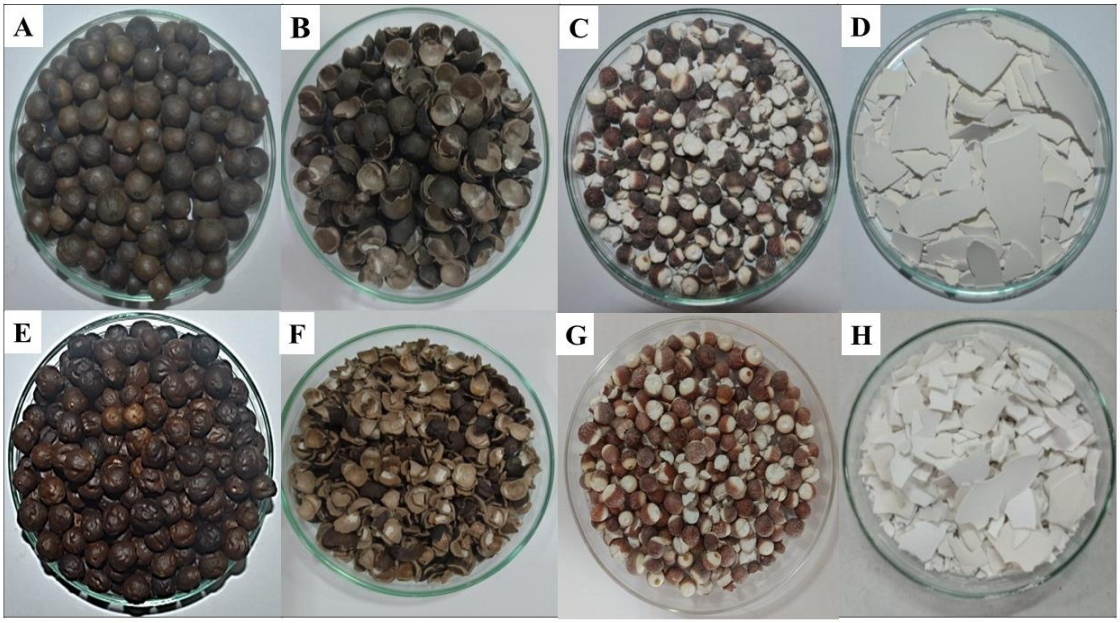

Fig. 2.2 (A) *Euryale ferox* seeds of Assam; **(B)** *Euryale ferox* kernel of Assam; **(C)** *Euryale ferox* shell of Assam; **(D)** *Euryale ferox* kernel starch of Assam; **(E)** *Euryale ferox* seeds of Manipur; **(F)** *Euryale ferox* kernel of Manipur; **(G)** *Euryale ferox* shell of Manipur; **(H)** *Euryale ferox* kernel starch of Manipur

Table 2.1 Chemical composition and amylose content of *Euryale ferox* kernel starch

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)	Amylose (%)
EFKS-A	10.70±0.20^{a}	0.72±0.06^{a}	0.24±0.03^{a}	0.26±0.03^{a}	0.24±0.08^{a}	36.13±1.45^{a}
EFKS-M	10.70±0.41^{a}	0.58±0.03^{b}	0.22±0.02^{a}	0.25±0.01^{a}	0.25±0.11^{a}	40.53±1.81^{b}

Values are represented as mean of triplicates ±SD; Values with different letters in the same column differ significantly ($p<0.05$)**;** EFKS-A: *Euryale ferox* kernel starch-Assam; EFKS-M: *Euryale ferox* kernel starch-Manipur

The proximate composition and amylose content of the EFKS are presented in Table 2.1. The moisture content of EFKS-A and EFKS-M were 10.70 and 10.63% respectively. Moisture content should fall within the recommended moisture content (< 13%) for long term safe storage [25, 27, 35]. The ash level of EFKS was minimal and within the required limit (0.5%) for grade-A industrial starches [26]. The ash content of *Euryale ferox* kernel starch isolated from two different cultivars was in the range of 0.25–0.26g/100 g. The low-fat content of raw seed of *E. ferox* (0.5%) might be responsible for the low-fat content of EFKS. The efficiency of the starch isolation procedure may also play a role in the purity of the isolated starch. The botanical origin, weather conditions and environmental factors, farming techniques, and the procedure used for starch isolation are some of the factors affecting the chemical composition of starch [2, 28, 35]. The amylose content of EFKS-A and EFKS-M obtained in this study were 27.13% and 26.53%, respectively. Amylose content of 26.69 and 27.29% has been reported from two *Euryale ferox* cultivars grown in China [39]. The amylose content of starch sample may differ even among the crop of same species. Amylose content has influenced on the physicochemical, functional, and nutritional properties of starch. The amylose fraction of starch occupies the amorphous region of starch granules; hence higher amylose content indicates low crystallinity and low swelling capacity [28, 38]. The disparity in chemical composition of two *E. ferox* cultivars could be related to varietal difference, level of maturity, environmental and climatic conditions [30, 32].

2.3.3 Morphology of *Euryale ferox* kernel starch

Euryale ferox kernel starch (EFKS) exhibited polyhedral, angular and oval shape with remarkably small granule size of 1~3μm. There was no evidence of holes, or cracks on the exterior surface of the granule. *Euryale ferox* kernel starch granules were exceptionally smaller than most of the conventional starches *viz*., potato and maize starch. *Euryale ferox* kernel starch granules is comparable to rice starches (2–8 μm), the smallest granules starch among industrially utilized starches [8]. The size and morphology of *Euryale ferox* kernel starch granules obtained from SEM micrograph (Fig. 2.3) are in agreement with the previous findings [39]. The shape, size, and arrangement of starch granules has influenced on the physicochemical properties of starch such as the water absorption capacity, swelling capacity and gelatinization temperature of the starch. Normally, the morphology of starch

granules is mostly determined by their botanical origin, starch granule production by the plant as well as plant's physiology, amylose content etc. [14, 18, 23, 30, 33, 35].

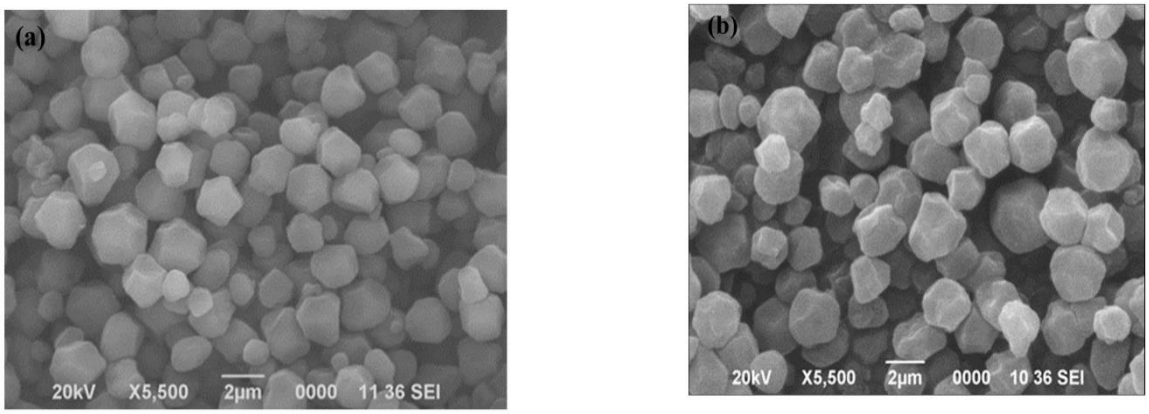

Fig. 2.3 (a) SEM micrograph *of Euryale ferox* kernel starch of Assam; **(b)** SEM micrograph of *Euryale ferox* kernel starch of Manipur

2.3.4 X-ray diffraction pattern

Starch can present a clear cut XRD pattern, and starch obtained from different sources exhibit different XRD pattern [22, 32]. The XRD spectra of *Euryale ferox* kernel starch have been illustrated in Fig. 2.4 and the two diffractograms had the same profiles. Strong diffraction peaks at around 15º and 23º, and a joint peak at 17º and 18º of 2θ were seen in the XRD pattern of *Euryale ferox* kernel starch. The XRD pattern exhibited by *Euryale ferox* kernel starch were compared with the standard diffraction patterns [40], and found that *Euryale ferox* kernel starch displayed A-type X-ray diffraction pattern which is generally seen in cereal starches. Similar XRD pattern of EFKS has been reported from Chinese cultivars [39]. Cassava, colocasia and arrowroot are some of the non-cereal starches which displays A-type X-ray diffraction pattern (10; 25]. The degree of crystallinity of EFKS-A and EFKS-M were 35.39% and 43%, respectively. The amylose content was negatively correlated with the degree of crystallinity. XRD pattern of a starch has influenced on the water absorption capacity, gelatinization temperature, retrogradation tendency and digestibility of starch [18].

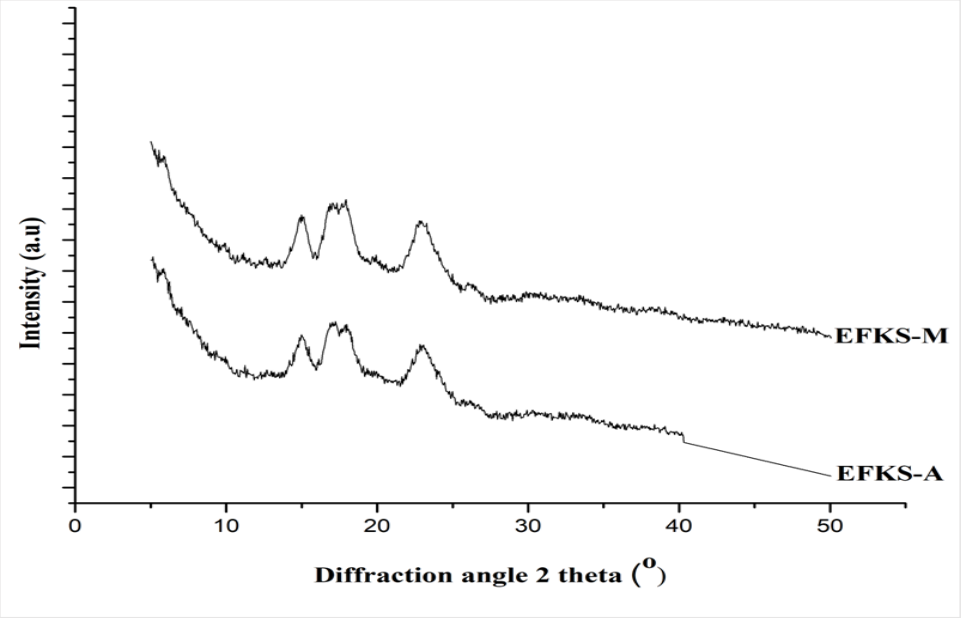

Fig. 2.4 X-ray diffraction (XRD) pattern of *Euryale ferox* kernel starch-Assam (EFKS-A); *Euryale ferox* kernel starch-Manipur (EFKS-M)

2.3.5 Fourier transform infrared spectroscopy analysis (FT-IR)

FT-IR spectra of the *Euryale ferox* kernel starch samples are illustrated in Fig. 2.5. A broad band at around 3000-3600 cm^{-1} was observed. The absorption peak at 3377-3392 cm^{-1} was due to the stretching vibration of free and bound O-H group [9]. The peaks at 2930-2937 cm^{-1} were arising owing to CH_2 stretching vibration of the glucose [29]. The sharp absorptions band observed at 1641-1648 cm^{-1} was due to H-O bending vibration [7]. The absorptions band at 1417-1424 cm^{-1} was produced on account of angular twisting of C-H [38]. The peak observed at 1148-1156 cm^{-1} was attributed to the conjoining of C–C and C–O bond stretching [38]. The peaks at 1082 cm^{-1} and 1022 cm^{-1} were ascribable by the anhydro glucose ring O-C stretch [7]. The characteristics peaks appeared between 933 and 1156 cm^{-1} were linked with C-O bond stretching [7]. Peak observed at 851-858 cm^{-1} was due to CH_2 deformation and the absorption band at 537 cm^{-1} was due to the skeletal modes of pyranose ring [20]. The absorption peak at 769 $cm^{-1}$was attributed to C-C stretching [9]. These entire characteristics peak verified the carbohydrate nature of the samples.

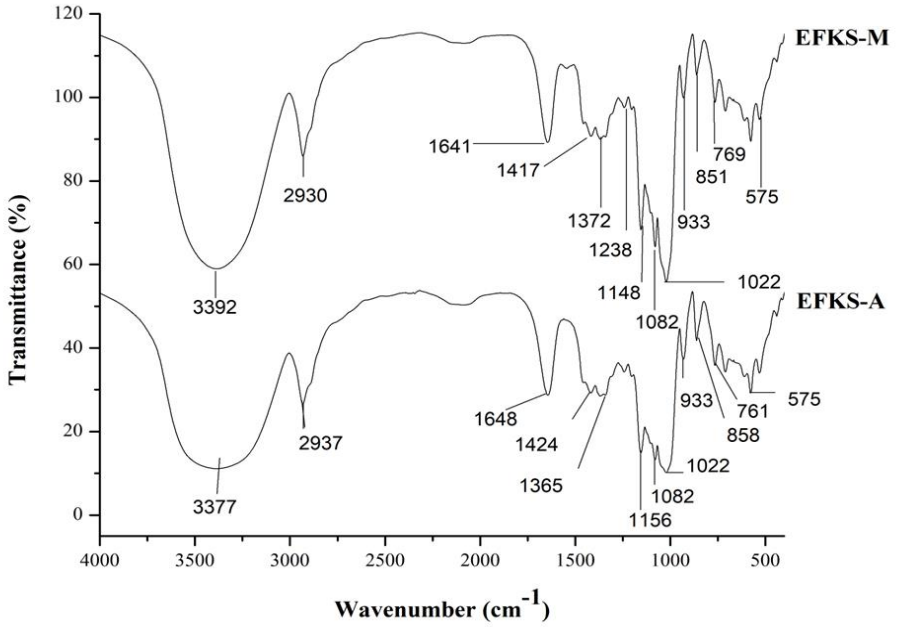

Fig. 2.5 FT-IR spectra of *Euryale ferox* kernel starch-Assam (EFKS-A); *Euryale ferox* kernel starch-Manipur (EFKS-M)

2.3.6 Gelatinization properties

The gelatinization temperature of *Euryale ferox* kernel starch is presented in Table 2.2. It was observed that *Euryale ferox* kernel starch of Manipur has exhibited higher gelatinization temperature and higher gelatinization enthalpy than *Euryale ferox* kernel starch of Assam. The high gelatinization properties of *Euryale ferox* kernel starch may be attributed due to its high amylose content, small granule size, and lower swelling capacity etc.

Table 2.2 Gelatinization properties of *Euryale ferox* kernel starch

Sample	To(°C)	Tp (°C)	Tc (°C)	ΔH (°C)
EFKS-A	65.20±1.10[a]	94.60±1.52[a]	106.80±1.25[a]	11.8±0.06[a]
EFKS-M	65.70±1.00[a]	98.30±1.50[b]	109.60±2.05[a]	12.4±0.08[a]

Values are represented as mean of triplicates ±SD; Values with different letters in the same column differ significantly ($p<0.05$); To: Onset temperature; Tp: Peak temperature; Tc: Conclusion temperature

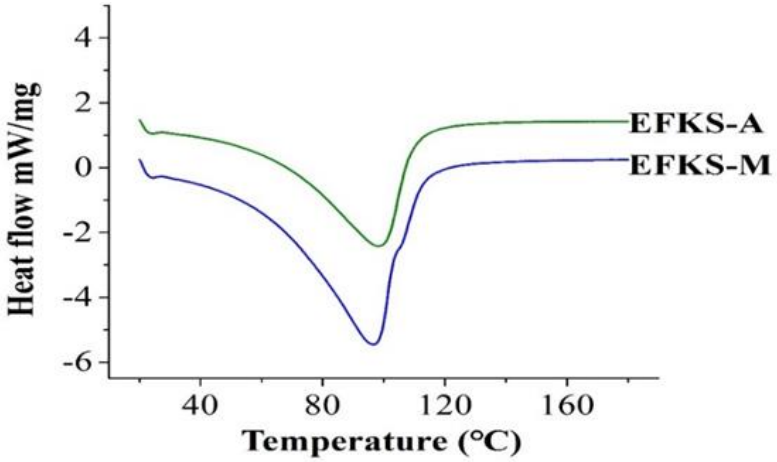

Fig. 2.6 Gelatinization properties of *Euryale ferox* kernel starch-Assam (EFKS-A); *Euryale ferox* kernel starch-Manipur (EFKS-M)

2.3.7 Thermogravimetric analysis

The thermogravimetric curves corresponding to the thermal stability were shown in Fig. 2.7. The thermal decomposition pattern of *Euryale ferox* kernels starch obtained from the TGA curve showed that there were three distinct weight losses. The initial onset thermal decomposition happened in the temperature period of 30–280°C, which is associated with the vaporization of absorbed water. The next stage weight loss occurred at 280–345°C occurred due to pyrolysis of starch. The third stage weight loss occurred at around 345°C was due to the degradation of the starch backbone.

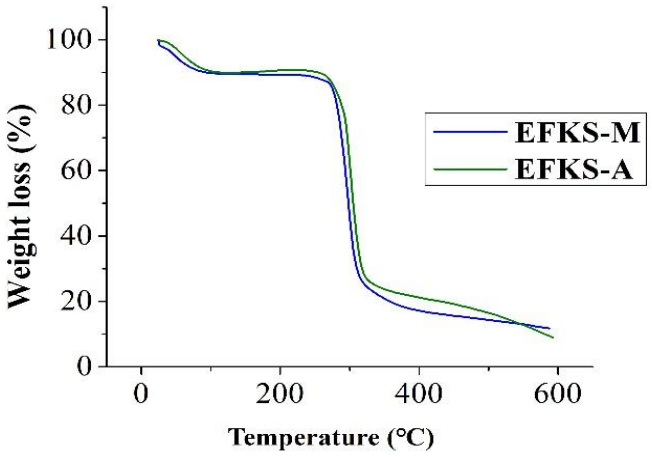

Fig. 2.7 Thermal degradation pattern of *Euryale ferox* kernel starch-Assam (EFKS-A); *Euryale ferox* kernel starch-Manipur (EFKS-M)

2.3.8 Swelling power and solubility index

Swelling power is the quantity of water sucks up by starch granules, whereas solubility is the amount of starch dissolved during the swelling process [16, 17]. The swelling power and solubility of starch are due to interactions between water molecules and starch chains in amorphous and crystalline portions of the starch [28, 37, 30]. From the swelling pattern, we can summarize that EFKS resist swelling at lower temperature, presumably ascribable to its high initial gelatinization temperature. Rise in swelling power of EFKS was observed from 70ºC and above.

The swelling power of EFKS-M was higher than EFKS-A throughout the range of temperatures (60-90ºC). The highest swelling power of EFKS was observed at 90°C. The swelling power of EFKS-A and EFKS-M were 2.87 and 2.73 at 60°C and finally increased up to 12.03 and 9.57 g/g at 90°C, respectively. Swelling capacity of similar pattern has been reported for legume starches [2]. When starch granules are cooked in surplus water, the hydrogen bond that holds the intermolecular packing of amylose and amylopectin starts to disintegrate in the amorphous area and spreads to the crystalline region, causing starch granules to swell and some soluble starch to leach [15, 16, 24]. Amylopectin is responsible for swelling power whereas amylose inhibits swelling, as a result starch with higher percentage of amylose is better reinforced and displayed low swelling capacity [32, 38]. Moreover, the small size of EFKS granules might be responsible for its low swelling capacity [36]. Considering all these contributing aspects such as high amylose content, and small granule size of EFKS might be responsible for its low swelling capacity.

Table 2.3 Swelling capacity and Solubility of *Euryale ferox* kernel starch

Parameters	Sample	Temperature (°C)			
		60	**70**	**80**	**90**
Swelling capacity	EFKS-A	2.87±0.38[a]	5.01±0.46[a]	5.53±0.43[a]	12.03±0.82[a]
	EFKS-M	2.73±0.30[a]	3.78±0.33[b]	4.76±0.42[a]	9.57±0.98[b]
Solubility	EFKS-A	1.58±0.09[a]	3.93±0.20[a]	7.63±0.46[a]	9.23±1.12[a]
	EFKS-M	1.17±0.03[a]	3.12±0.06[a]	6.23±0.35[a]	8.48±0.44[a]

Values are represented as mean of triplicates ±SD; Values with different letters in the same column differ significantly ($p<0.05$); EFKS-A: *Euryale ferox* kernel starch-Assam; EFKS-M: *Euryale ferox* kernel starch-Manipur

The pattern of starch swelling power and solubility of EFKS followed the same trend. The solubility of EFKS-A and EFKS-M was 1.58 and 1.17% at 60°C and gradually increased up to 9.23 and 8.48% respectively when the suspension reached temperature of 90°C. During the process of heating of starch suspension, we could see rise in solubility with the increase in temperature because of the swelling of starch granules which causes amylose to leach out and it was supported by the findings of other researchers [28]. Previous studies reported that the solubility of *Euryale ferox* kernel starch was > 5% at 80℃ [39]. The disparity in solubility index of starch from the same species arises due to the differences in level in maturity, variety, topographical conditions, agronomic practices, procedure employed for starch isolation, chain length, and ratio of amylose and amylopectin etc. The variation in solubility among starches is attributed due to the differences in rate of disintegration of starch granules, heterogeneity in chain length and proportion of amylose and amylopectin fractions, granule morphology, and lipid content among others [17, 32, 38].

2.3.9 Pasting properties

Table 2.4 Pasting properties of *Euryale ferox* kernel starch

Sample	Pasting Temperature (°C)	Peak Viscosity (cP)	Hold Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)
EFKS-A	78.81±0.7^{a}	2734±20^{b}	2485±25.0^{b}	249±4.5^{b}	3078±22^{b}	593±47^{b}
EFKS-M	83.36±0.85^{b}	1953±17^{a}	1901±14.0^{a}	52±3.00^{a}	2422±22^{a}	521±8^{a}

Values are represented as mean of triplicates ±SD; Values with different letters in the same column differ significantly ($p<0.05$)

The pasting parameters obtained from RVA amylograph revealed information about the changes in starch behavior during heating and the retrogradation tendency on cooling [38]. The pasting properties obtained from RVA of EFKS (Table 2.4) have shown that with the increase in temperature, starch slurry increased its viscosity due to removal of water from the leached-out amylose during starch swelling procedure [15,31].

The pasting temperature of EFKS-M (83.36°C) was higher than EFKS-A (78.81°C). The lowest temperature required to cook a sample is known as the pasting temperature [22, 27]. The pasting temperature (Tp) exhibited by EFKS were comparable with cereal starch such as rice (89.2°C), wheat (85.5°C); and maize starch (81.1°C) but higher than the tuber starch such as tapioca (69.5°C) and potato starch (65.5°C) [8].

The peak viscosity of EFKS-M (2734cP) was higher than that of EFKS-A (1953cP). Peak viscosity is the equilibrium point where there is stability between the swollen yet intact granules and leached out amylose in the suspension [2]. The lower PV of EFKS-A than EFKS-M might be due to controlled swelling of starch granules due to higher amylose starch [17, 22]. The hold viscosity of EFKS-A and EFKS-M were 1901cP and 2485cP, respectively. After attaining peak viscosity, starch granules were continuously heated at high temperature (95°C), the swollen granules were broken and leached amylose, thus decrease in the viscosity [22]. The breakdown viscosity of EFKS-A and EFKS-M were 52cP and 249 cP, respectively, were comparable to tuber starch (*Dioscorea alata* Linn) [18]. The extent of disintegration of swollen starch granules is known as breakdown viscosity which is the function of rigidity of the swollen starch granules [4].

The final viscosity of EFKS-A and EFKS-M were 2422cP and 3078cP, respectively which was comparable with normal maize starch (3157cP); and wheat starch (3272cP), remarkably higher than waxy maize starch (1053cP), and tapioca starch (1437cP) [8]. The final viscosity of starch is determined by the strength of the cooled, cooked paste under low shear [8]. The aggregation of amylose molecules is responsible for the rise in ultimate viscosity [38].

The setback viscosity of EFKS-A and EFKS-M were 521cP and 593cP which was comparable to tapioca starch (551cP), but extremely lower than normal maize starch (1308cP) [8]. EFKS possessing low setback viscosity exhibit higher resilience against retrogradation [4]. The rise in viscosity appeared due to the rearrangement of amylose molecules that have been leached from swollen starch granules during the process of cooling is normally considered to assess starch gelling capability or retrogradation tendency [38, 24]. Long chain amylopectin and small amylose molecules are usually retrograded quickly; therefore, the structure and chain of amylose and amylopectin is responsible for the gelling capacity or retrogradation susceptibility [18]. The low

breakdown viscosity of EFKS indicates high stability to remain intact at high temperature [33].

The high pasting temperature of EFKS is attributed by its high amylose content which rendered better resistant against rupture, resulting low water absorption capacity and low swelling capacity [5, 30]. The low swelling power of EFKS was supported by its low peak viscosity, hold viscosity, final viscosity and setback viscosity of starch [30]. Considering pasting parameters as revealed by RVA, we can conclude that this starch will be suitable for food products requiring high processing temperature and alteration in viscosity is unwanted in the course of heating and cooling, suggesting its possible application such as canned foods, baby food, sauces, bread etc. [13, 15, 33]. The origin of starch, granule size, rigidity of starch granules against swelling, amylose concentration, and chain length distribution of amylopectin are all factors that can affect the starch pasting properties [32].

2.3.10 Color characteristics of *Euryale ferox* kernel starch

Color is often the first criterion which plays an important role on the acceptability of a food product. The color characteristics of the EFKS samples are shown in Table 2.5. Higher lightness values and low values of protein, ash, and fat exhibited by EFKS reflect higher degree of whiteness and purity. "L*" value higher than 90 indicates high level of purity [2]. The high L* values together with its low chroma of redness and greenness of EFKS assured that it could successfully replace corn or rice starch in applications requiring color transparency such as edible packaging films and coating. Color of native starch may be influenced by genetic makeup, granule morphology, endogenous polyphenols, ascorbic acid and pigments presents in the starch source [1].

Table 2.5 Color parameters of *Euryale ferox* kernel starch

Sample	L*	a*	b*
EFKS-A	93.67 ± 0.60^{a}	1.05 ± 0.15^{a}	4.96 ± 0.21^{a}
EFKS-M	97.10 ± 0.67^{b}	0.53 ± 0.11^{b}	1.40 ± 0.32^{b}

Results are mean of triplicates ±SD; Values with different letters in the same column differ significantly ($p<0.05$)

2.3.11 *In vitro* starch digestibility

Table 2.6 *In vitro* starch digestibility of *Euryale ferox* kernel starch

Sample	Native starch			Retrograded Starch		
	RDS %	**SDS %**	**RS %**	**RDS %**	**SDS %**	**RS%**
EFKS-A	83.47±1.10[a]	11.60±0.85[a]	4.93±1.16[a]	77.67±1.01[b]	15.63±0.96[a]	6.7±1.24[a]
EFKS-M	81.12±0.64[a]	12.63±0.85[a]	6.25±1.21[a]	74.37±1.00[a]	15.23±0.81[a]	10.40±1.75[b]

Results are mean of triplicates ±SD**;** Values with the different letters in the same column differ significantly ($p<0.05$)

The *in vitro* starch digestibility of native, and retrograded EFKS is presented in Table 2.6. In native EFKS, RDS accounted for 81.12-83.47% whereas SDS accounted for 11.60-12.63%. RS content of EFKS-A and EFKS-M was 4.93% and 6.25%, respectively in its native form. RS content of native EFKS was found to be higher than most of the cereal starches but comparable with legume starches. RS content of 60-65% in native pigeon pea starch has been reported [19]. EFKS-A possessing higher amylose content exhibiting lower degree of crystallinity, contained higher proportion of RS than EFKS-M. The disparity in the *in-vitro* starch digestibility between *Euryale ferox* starch cultivars was due to the due to the interaction of various contributing elements such including botanical origin, granule morphology, proportion of amylose and amylopectin, molecular arrangement of amylopectin, and crystallinity [14]. The double helical structure of the amylose molecule makes high amylose starch difficult for digestive enzymes to access [33]. Whereas, retrograded EFKS had higher RDS and lower RS than native EFKS. A similar trend has been reported for tuber starch [14]. Gelatinization disturbs the intermolecular and intramolecular hydrogen bonds within starch chains, letting the granules to bulge and dissolve, making the starch easily digestible. In addition, during the process of retrogradation of gelatinized starch, the amylose chains reassociate to assemble the double helices network, and the amylopectin reassemble to form crystallites, resulting in decreased receptivity against enzymes responsible for starch digestion [6]. Based on our findings, we concluded that starch from retrograded EFKS would make an excellent RS material.

2.4 Conclusion

There were disparities in the physicochemical, functional, and nutritional properties of *Euryale ferox* kernel starch of Assam and Manipur Regions of India. Due to its small granule size, EFKS has also the potential to cater the ever-increasing demand of small granule starch for food and non-food applications without further modification. Small granules can be used as a filler material for biodegradable packaging films, as well as for flavor encapsulation. Moreover, small granules starches are highly demanded because of their glossy creamy texture that can be utilized as fat replacers in reduced calories food products. EFKS displayed unique physicochemical and functional properties, possessing high pasting temperature, and low viscosity suggesting its possible applications in food products requiring high temperatures to cook.

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