



Quasi Emulsion Solvent Diffusion Modification of Underutilized *Chenopodium album* Starch and its Characterization

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The present study was designed to investigate the changes occur on *Chenopodium album* starch (CAS) after modification through quasi emulsion solvent diffusion (QESD) method. Modification of starch was carried out by HCl (0.1, 0.25, 0.50, 0.75 and 1M) followed by precipitation. The QESD modification significantly reduced the amylose content, water absorption capacity, oil absorption capacity, solubility as well as swelling power of treated starch. Gelatinization behaviour of modified starch showed an increase in pasting temperature. The SEM images showed the formation of regular and spherical shaped larger starch particles. There was an improvement in crystallinity from 20.01 to 29.86% after modification as shown in X-ray analysis. Overall, it indicates that QESD treatment results into formation of spherical crystalline agglomerates.

Keywords: Quasi emulsion solvent diffusion, *Chenopodium album*, Flow properties, Starch.

INTRODUCTION

Nowadays, industries demand specific and customized starch, to reduce processing losses and to ensure the high-quality productions [1]. In industrial unit operations, such as filling, compression and transportation, the flow properties of starch are of great concern [2]. In this regard, for any industry, the properties of powdery raw materials cannot be ignored as these are influenced by the types of suppliers, composition, geographical variations, humidity, processing techniques, morphology and particle size distribution. All these properties may significantly affect the resulting size, crystallinity and process behaviour. Various studies have been performed to modify starches to alter their physico-chemical and flow properties such as cohesiveness, compressibility and caking strength [3]. These methods included pre-gelatinization, chemical modification like octenyl succinate anhydride (OSA), acetylation, annealing, acid hydrolysis, and physical modification like heat moisture treatment (HMT), etc. [4,5]. Out of different available starch modification methods, the spherical crystallization method is accepted among the industries due to efficient crystallization and particle size enlargement, resulting in better [6]. Depending on the technique of conversion, the characteristics

of the converted starches differ. Quasi emulsion solvent diffusion (QESD) modified starches have been developed to overcome the drawbacks of native starches (water repellence, insolubility or failure of granules to swell and develop viscosity in cold water, excess or uncontrolled viscosity after cooking and so on, extending the starch's utility in a variety of industrial applications [6]. Native starch was initially changed to increase its functioning for industrial purposes and therefore broaden its use, allowing it to be cooked at higher concentrations than the native substance. These changes entail the chemical or physical manipulation of the starch granule to produce rupturing of some or all of the starch molecules, weakening them and reducing their ability to expand during pasting. As a result, the viscosity of the sol generated is reduced, allowing the final sol to be distributed at larger concentrations. The spherical crystallization consists of three methods viz. spherical agglomeration, ammonia diffusion (AD) and quasi-emulsion solvent diffusion (QESD). The initial crystallization in all these processes involves dissolving the substance of interest in a solvent and then combining it with an anti-solvent. The primary mechanisms involved during crystallization techniques are: (i) Flocculation, in which bridging solvent displaces the liquid from the crystal region's surface and these crystals are taken by agitation into

proximity and (ii) zero growth zone, in which trapped fluid is squeezed out during the growth process, followed by the bridging solvent onto the surface of small flocks [7].

These mechanisms give the nucleus plasticity and spherical shape to the granules with increased crystallinity, resulting into better flowability. Therefore, the present study was undertaken to modify the *Chenopodium album* starch using QESD method for better flowability.

EXPERIMENTAL

Chenopodium album (variety: Pusa 1) grains were obtained from the Indian Agriculture Research Institute (IARI), New Delhi (India). These grains were ground using a mixer grinder and passed through a 60 BSS sieve. Sigma-Aldrich (India) provided the other AR Grade chemicals which were utilized in the investigation.

Isolation of starch: The *Chenopodium album* starch was isolated using alcohol aided alkaline method as described by Singh *et al.* [8] and dried in a hot air oven at 45 °C for 24 h.

QESD modification of starch: Starch sample (50 g) was dissolved in 2000 mL of HCl (0.1, 0.25, 0.5, 0.75 and 1M) for a minute at 40 °C. Further, the process was followed by addition of ethanol (200 mL) for precipitation of spherical crystalline starch. These precipitated crystals were collected and dried in an oven for 24 h at 40 °C and further kept in a desiccator. The dried crystals of starch were passed through a 100 BSS sieve, packed in aluminum pouches and stored in a desiccator at room temperature.

Functional properties: Amylose content (AC) (%) was determined by the method of Morrison & Laignelet [9]. 1N NaOH (9 mL) and 1 mL 95% ethanol were added to the starch sample (50 mg) in a 100 mL volumetric flask. Now, 5 mL aliquot, 1 mL 1N acetic acid and 2 mL iodine solution were added to another 100 mL volumetric flask. After 20 min, the absorbance of samples was measured by spectrophotometer (Cole-Parmer, Germany) at 620 nm. The amylose content was calculated using the standard amylose solution standard curve.

Oil and water absorption capacity were determined according to the method of Singh *et al.* [8]. In a weighed centrifuge tube, 15 mL of purified water was added to 1 g of starch sample. The tube was agitated for 2 min and centrifuged at 6000 rpm for 20 min. Oil absorption capacity (OAC) was expressed as the weight of 1 g of dried sample bound by 1 g of oil. For determining oil absorption capacity (WAC), water was used in place of water.

The starch's swelling power was determined using the procedure of Perez *et al.* [10] with slight modification. In brief, starch slurry (2 g dry starch /100 mL water) was prepared in centrifuge tubes. The slurry was heated at 55, 65, 75, 85, 95 °C for 30 min followed by cooling at room temperature. After cooling, the tubes were centrifuged at 5000 rpm for 20 min.

Solubility and swelling power were determined using eqns. 1 and 2, respectively.

$$\text{Solubility} \left(\frac{\text{g}}{100 \text{ g}} \right) = \frac{\text{Weight of solubles (g)}}{\text{Weight of sample (g, dry basis)}} \times 100 \quad (1)$$

$$\text{Swelling power} \left(\frac{\text{g}}{\text{g dry basis}} \right) = \frac{\text{Weight of sediment paste (g)}}{\text{Weight of sample} \times (100 - \text{WSI})} \quad (2)$$

Colour values: A portable chroma meter (CR 400, Konica Minolta, Japan) was used to measure the color values of native and modified starch. The color was measured in triplets to get L*(lightness), a*(greenness to redness) and b*(blueness to yellowness) values.

Pasting properties: Pasting properties were analyzed using Rapid Visco Analyzer (RVA Tecmaster, Australia). Pasting characteristics were analyzed through visco-amylogram in terms of peak viscosity, total set back, breakdown and final viscosity [11].

FTIR spectroscopy: Functional group analysis of samples was conducted at room temperature using the FTIR spectrometer (ALPHA Bruker, Germany). The attenuated total reflection (ATR) plate was cleaned with isopropyl alcohol, and scanning was done in range of 4000-400 cm⁻¹.

X-Ray diffraction analysis: An X-ray diffractometer was used to examine the X-ray diffraction patterns of starch samples (Ultimate IV X-Ray Diffractometer, Rigaku, Japan) operated at 40 kV, 30 mA over the 2θ range between 10-50°, using radiation wavelength of 1.5405 Å.

Morphological properties: Morphology of starch was conducted by Scanning Electron Micrography (SU8020, Hitachi Int., Japan). Starch samples were photographed by SEM at 5 kV acceleration voltage with 5000x magnification.

Thermal properties: Thermal properties were determined using differential scanning calorimetry (DSC, NETZSCH, Germany). The hermetically sealed pans of starch were heated over the range of 20 to 200 °C (10 °C/min) with a reference of empty aluminium pan.

Statistical analysis: Using SPSS (ver.25, IBM software, USA), one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) were applied to find significant differences.

RESULTS AND DISCUSSION

Functional properties: Amylose content of native and modified starch differed significantly ($p < 0.05$) with higher mean values in native starch (14.74%) and lower in modified starch (12.95%) at 1 M HCl (Table-1). Amylose exists preferentially in the amorphous regions. Therefore, amylose molecules were fragmented more quickly than amylose-pectin molecules. The decline in the amylose content in modified starch may be due to the fact that HCl preferentially targets the amorphous regions during hydrolysis [12,13].

The values of water absorption capacity (WAC) were found higher for native than modified starch but a reverse effect was observed in case of oil absorption capacity (OAC) (Table-1). The low WAC with increasing concentration of HCl in starch samples might be due to the reduction of the amorphous region in the starch granules and increased alkenyl group attachment which in turn decreased the amount of water binding sites and makes the starch more hydrophobic. Similar decrease of WAC and increase of OAC were reported by few researchers [14,15] for chemically modified corn and chestnut starch, respectively.

TABLE-1
EFFECT OF QUASI EMULSION SOLVENT DIFFUSION MODIFICATION ON
PHYSICO-CHEMICAL PROPERTIES OF *Chenopodium album* STARCH

Properties	Acid treatment (M HCl)						
	Native	0.1	0.25	0.50	0.75	1.0	
Amylose content (%)	14.74 ± 0.07 ^a	14.49 ± 0.15 ^{ab}	14.14 ± 0.69 ^b	13.51 ± 0.12 ^{cd}	13.56 ± 0.31 ^d	12.95 ± 0.09 ^c	
WAC (%)	175.00 ± 3.00 ^a	156.00 ± 4.58 ^b	151.33 ± 3.51 ^{bc}	158.33 ± 3.51 ^b	148.33 ± 3.06 ^c	125.67 ± 4.93 ^d	
OAC (%)	222.00 ± 3.61 ^a	124.33 ± 4.04 ^b	120.33 ± 2.52 ^b	117.00 ± 4.58 ^b	114.67 ± 5.51 ^b	104.67 ± 9.87 ^c	
Color value	L	96.02 ± 0.41 ^a	95.693 ± 1.21 ^a	96.15 ± 0.01 ^b	95.68 ± 0.15 ^a	95.64 ± 0.09 ^b	95.43 ± 0.07 ^b
	a	-1.01 ± 0.03 ^{ab}	-0.89 ± 0.01 ^{ab}	-0.91 ± 0.005 ^b	-0.87 ± 0.05 ^d	-1.00 ± 0.005 ^c	-0.95 ± 0.01 ^c
	b	3.38 ± 0.13 ^c	4.05 ± 0.0 ^a	3.91 ± 0.04 ^a	3.38 ± 0.12 ^d	3.18 ± 0.02 ^b	4.20 ± 0.01 ^b

The values are mean ± standard deviation of three replicates. Values with different superscript in a row (effect of acid concentration) are significantly different ($p < 0.05$). (WAC-Water absorption capacity; OAC-Oil absorption capacity; Plywood- Parallel, Plywood*- anti parallel, GIS-galvanized iron sheet).

Both swelling power and solubility of native starch were found higher as compared to modified starch. In case of modified starch, the solubility and swelling power decreased with the increasing concentration of acid as shown in Figs. 1 and 2. However, solubility and swelling of all starches were improved as the temperature of swelling and solubility arrangement increased from 55 °C to 95 °C. The lowest solubility and

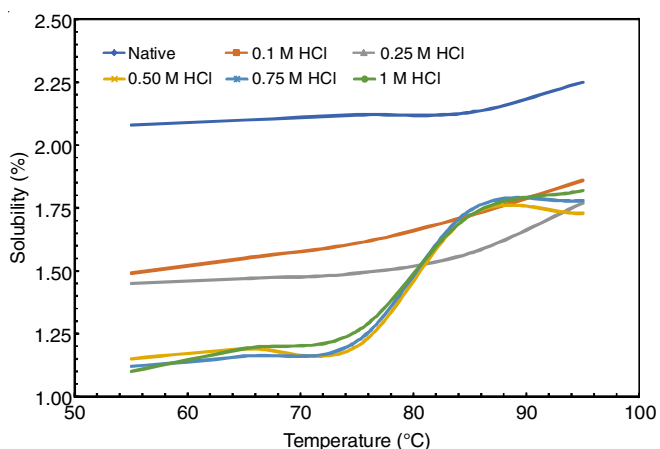


Fig. 1. Effect of quasi emulsion solvent diffusion modification at different acid concentrations on solubility of starch

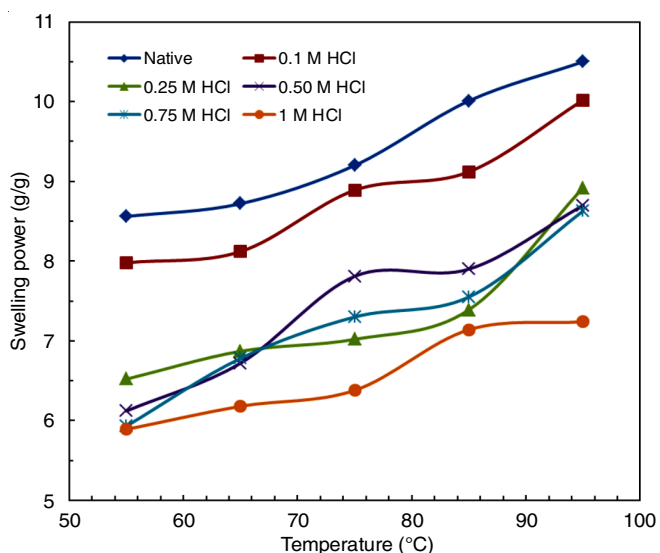


Fig. 2. Effect of quasi emulsion solvent diffusion modification at different acid concentrations on swelling power of starch

swelling were found for starch modified with 1 M HCl. This might be attributed to the temperature used, which was far above the temperature of *Chenopodium album* starch's gelatinization. It may be due to the fact that hydrogen bonds begin to disturb as the temperature of an aqueous starch suspension starts elevating above the gelatinization level, followed by the binding of water molecules to freed hydroxyl group, resulting in continuous bonding. The similar results were observed in another study [16], wherein the modified corn starch heated above the gelatinization temperature resulted in exposure of amylopectin and crystalline structure resulting in low solubility of starch granules.

Colour values: QESD modification significantly ($p < 0.05$) affected the colour values of native and modified starch as shown in Table-1. The modification significantly decreased the 'L' value (lightness) from 96.02 to 95.43 for native and modified starches, respectively. On the other hand, the 'a' value (redness) was found maximum for 1 M and minimum for native starch (-1.01). The b value of native starch was found lower as compared to modified starch which signifies more yellowness for modified starch as compared to native starch. The colour values of modified starch were found similar to chemically modified maize starch after treatment with HCl [17]. The reduction of lightness might be due to hydrolysis of starch. In another study, for corn starch, Boudries *et al.* [18] acknowledged that L* values greater than 90 granted an acceptable whiteness limit for starch purity. In this study, the color values of modified starches were observed under the acceptable whiteness category.

Pasting properties: The pasting properties of native and modified *Chenopodium album* starch are shown in Table-2. The peak temperature (PT) of native (80.05 °C) and modified starch (78.30-81.45 °C) were found to be comparable except for 1 M treated sample in which PT drastically increased to 87.20 °C. The increase in PT at higher acid concentration might be due to severe alteration after QESD modification. The similar increase in the pasting temperature was observed by Lawal [19] in succinyl and acetyl starch derivatives of hybrid maize, where both derivatives showed improved ordered alignment with decrease in stability. Unlike peak temperature (PT), peak viscosity (PV) of modified starch was greatly reduced with increase in the HCl concentration. The highest PV was observed for native starch (6565 cP) which was reduced to 490 cP for 1 M modified starch. The reduction in PV could be

due to the crystallization of starch and partial hydrolysis of amorphous region leading to restriction in starch granules to achieve their swelling capacity. This restricted swelling capacity of treated starch resulted into reduced peak viscosity. The decrease in breakdown viscosity (BV) after modification indicated higher aggregation potential and lower retrogradation tendency. The reduction in final viscosity (FV) and setback viscosity (SV) was observed with increasing concentration of HCl (up to 0.5 M). The reduction in FV and SV may be due to increase in breakage of starch chains and the association of starch amylopectin molecules. This consistency and resistance of starch granules to shear pressure and the retrogradation of leached amylose in modified starch paste were found to be comparable with the reported results [20] for *Rhizoma dioscorea* starch.

FTIR studies: Native starch showed (Fig. 3) five major peaks near 3844, 2890, 1693, 1146 and 1019 cm^{-1} . The wide band at 3844 cm^{-1} was commonly found due to stretch vibration of O-H. The peak at 2890 cm^{-1} corresponded to the protein and polysaccharide stretching vibrations of C-H. In addition, the absorption band at 1693 cm^{-1} represented the stretching

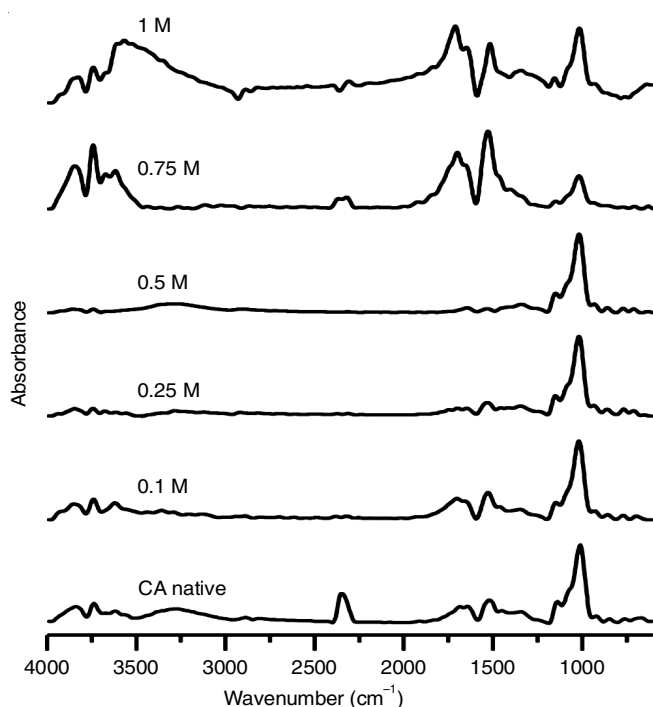


Fig. 3. FTIR spectra of native and modified *Chenopodium album* starch

vibration of the carbonyl bond (C=O), but according to the characterization of aromatic molecules, it was possibly caused by aromatic C-H bonds [12]. The bands varied between 1146 to 1019 cm^{-1} for C-O bonding.

A narrow range for wave shifting between 3844 and 3828 cm^{-1} was observed in modified starch. The stretching frequency at about 3400 cm^{-1} to 3200 cm^{-1} represented OH groups, which implied successful attachment of alcohol group after modification. Higher peak at 2886 cm^{-1} was due to symmetrical stretching of C-H group and bands appearing at 1709 cm^{-1} showed the vibrational mode of glycosidic linkage. Some peaks with decreasing molarity were found between 1508 cm^{-1} , which resemble the amorphous region of starch and water molecule having inter- and intramolecular hydrogen bonding. Modification of starch led to an increase in O-H bond stretching and the maximum was observed in 1M HCl treated starch. The frequent peak was observed between 1124 to 1087 cm^{-1} due to C-O vibrational stretching of alcohol at all concentrations of HCl in the modified starch.

XRD studies: The XRD pattern of native and modified starch showed typical A-type starch with strong peaks at 2θ about 15°, 17°, 18° and 23°, three different peaks at 2θ of 13° and 23°, and doublet at 16° and 17°. In modified starch, diffraction pattern also showed three different peaks at 17° and strong peak at 23° with duplet at 18° and 19°. Single peak occurred at 23° and the peak is turned into a shoulder at 18° (Fig. 4). No changes were found in the modified starch XRD pattern, which inferred no effects on the crystalline region due to modification.

The degree of crystallinity of native starch was increased from 20.01% to 20.66, 20.88, 24.32, 26.98, and 29.86% upon modification with increasing concentration of HCl. This might be due to the decrease of amorphous region as a result of interactions between amylose-amylose and amylose-amylopectin. Similar increase in crystallinity from 33.4 to 39.7% was found in corn starch after acid hydrolysis which attributed to decrease in amylose content due to preferential hydrolysis of amylose region of starch [21]. In another study, crystallinity was increased in pinhão starch from 26 to 27% after modification [22].

Morphological properties: As shown in Fig. 5, SEM of native and modified starch granules presented non-similar granular structures indicating that the modification altered shape and size of starch granules. Modified starch showed slightly smooth and spherical structural surfaces with losses in edge definitions after modification. These results are similar to the findings of Tapas *et al.* [23], which showed that spherically

TABLE-2
EFFECT OF QUASI EMULSION SOLVENT DIFFUSION METHOD ON
PASTING AND THERMAL PROPERTIES OF *Chenopodium album* STARCH

Samples	Pasting properties					
	Pasting temperature (°C)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
Native	80.05	6565	3045	3520	3882	837
0.1 M	81.45	1455	1329	126	1563	234
0.25 M	79.85	1198	1095	103	1299	204
0.50 M	78.30	890	743	147	852	109
0.75 M	79.85	861	573	288	1157	584
1.0 M	87.20	827	490	337	1036	546

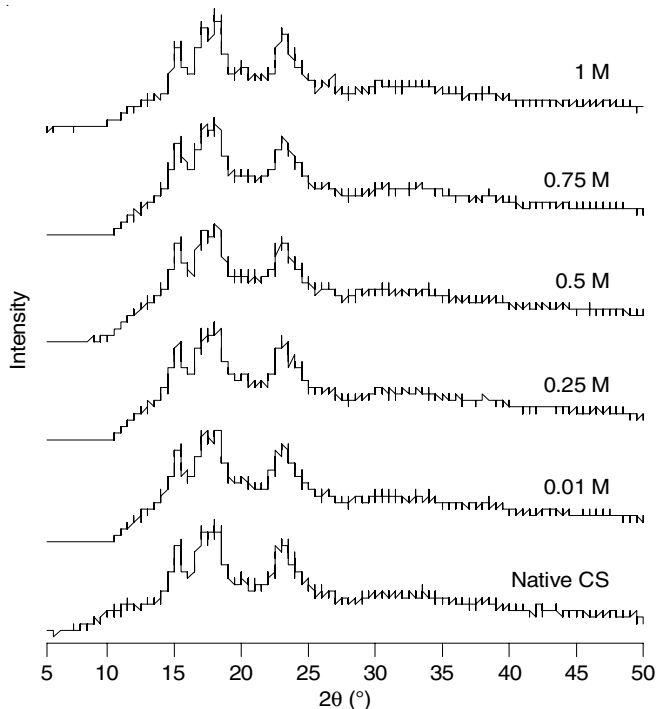


Fig. 4. Effect of quasi emulsion solvent diffusion method on X-ray diffraction pattern of *Chenopodium album* starch

altered morphological characteristics of starch granules lead to better flowability.

Thermal properties: Thermal parameters like onset temperature (T_o), mid temperature (T_m), end temperature (T_e) and enthalpy (H) of native and modified starch are illustrated in Table-3. Both native and modified starch showed two endothermic peaks (peak 1 and peak 2) in the temperature range of 42.9-63.9 °C (T_o), 45.7-66 °C (T_m), 45.9-68.1 °C (T_e) for peak 1 and 99.5 - 100.9 °C (T_o), 104-111.6 °C (T_m), 108-111.3 °C (T_e) for peak 2, respectively. In case of modified starch, endothermic peak shifted towards higher temperature with the increase in molar concentration. The second peak of thermogram in modified starch was also shifted to higher temperature from 99 -113 °C. Shifting of first and second peak in treated starch might be occurred because of increasing crystallization [24]. The shift in first and second peak may be due to the melting of complex amylose-pectin and non-complex crystalline amylose, respectively. Also, a wide peak for corn starch was reported by Lacerda *et al.* [24] around 100-150 °C with increased shift of endothermic peak.

Conclusion

In quasi emulsion solvent diffusion (QESD) treated starch, modification significantly reduced the amylose content, water absorption capacity, oil absorption capacity, solubility and swelling power of treated starch. The acidic solution acted as the solvent for the derivative and ensured uniform substitution of alcohol. Substitution of alcohol in starch chain, as confirmed by FT-IR analysis. This study showed that *Chenopodium album*

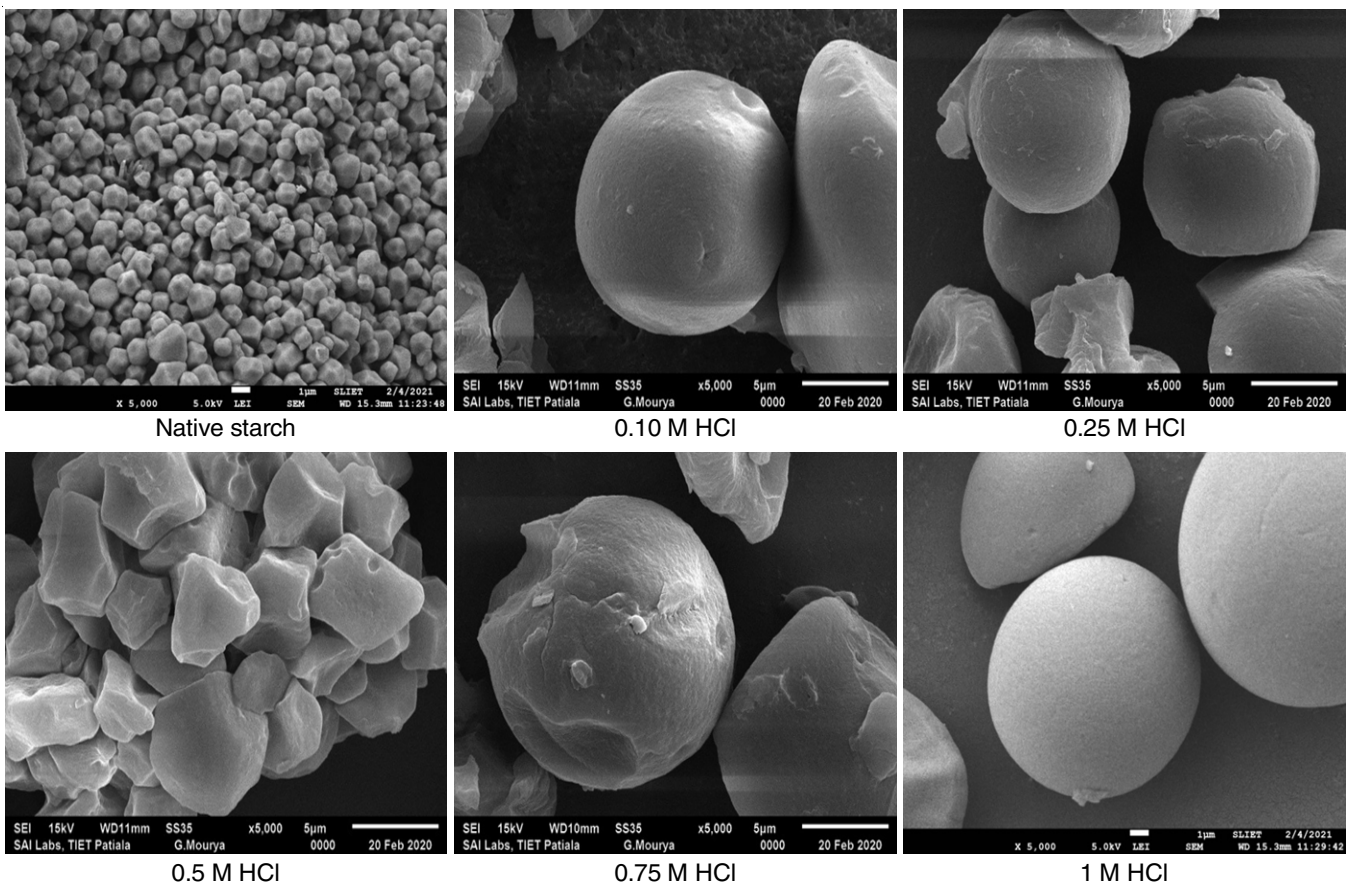


Fig. 5. Morphological properties of native and QESD modified starch at different levels of acid concentrations (5000x)

TABLE-3
THERMAL PROPERTIES (DSC) OF NATIVE AND QUASI EMULSION SOLVENT DIFFUSION MODIFIED *Chenopodium album* STARCH

Sample	Peak 1				Peak 2			
	T _o (°C)	T _m (°C)	T _e (°C)	ΔH (μV/mg)	T _o (°C)	T _m (°C)	T _e (°C)	ΔH (μV/mg)
Native	63.9	66.0	68.1	537.99	100.9	104.7	109.1	873.46
0.1 M	49.7	50.2	50.7	126.78	112.8	111.6	111.3	830.42
0.25 M	62.4	63.1	64.3	96.50	101.4	102.8	105.1	727.17
0.5 M	54.8	56.2	57.6	40.09	100.6	103.5	106.6	747.13
0.75 M	42.9	45.7	45.9	27.08	99.5	104.0	108.4	751.97
1.0 M	52.9	53.9	63.7	21.04	99.3	107.2	113.3	1323.83

*T_o = Onset temperature, T_m = Mid temperature, T_e = End temperature, ΔH = Enthalpy.

starch modified by QESD had better characteristics than native starch under 1 M HCl. It is concluded that under optimized condition *Chenopodium album* starch can be modified like other commercially available starch.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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