Research Article

Green Extraction and Carboxymethylation of *Amorphophallus* paeoniifolius Starch: A Novel Pharmaceutical Excipient

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Abstract

Background: Search for sustainable, biocompatible pharmaceutical excipients from underutilized agricultural resources is getting more momentum. Elephant Foot Yam (Amorphophallus paeoniifolius)—starch is an under-exploited potential ingredient for pharmaceutical use due to its special physicochemical properties.

Objective: The aim of this study was to develop a green method for starch extraction from Amorphophallus paeoniifolius tubers with deep eutectic solvents (DES), and to investigate the physicochemical changes after carboxymethylation to evaluate for use in pharmaceutical applications.

Methods: Starch was extracted by deep eutectic solvent, which was composed of two components: choline chloride: glycerol (1:2 molar ratio). The carboxymethylation was achieved by etherification with monochloroacetic acid. The comprehensive characterization involved FTIR, XRD, DSC, TGA, SEM, determination of swelling power and degree of substitution.

Results: Extraction of the starch with DES produced a high purity starch content (94.2 ± 1.8%) of the granules with good granular integrity. The degree of substitution for carboxymethylation was 0.45 ± 0.03. FTIR revealed the incorporation of carboxylate group (1605 cm^{-1} and 1420 cm^{-1}). The XRD showed a reduction of the relative crystallinity (28.5% to 15.2%). Thermal analysis revealed an increase in the onset decomposition temperature from 295.2 to 315.4. Swelling power increased 77% (from 18.5 to 32.8 g/g) and solubility increased 133% (from 12.3 to 28.6%). The SEM showed a difference in surface morphology between the native granules and the modified granules, and a roughened surface with pores was observed.

Summary: DES extraction is considered as an environmentally friendly method for the extraction of high-purity A. paeoniifolius starch. Carboxymethylation also improves the physicochemical properties making CM-EFYS an interesting material for the pharmaceutical excipients market.

Keywords: Amorphophallus paeoniifolius, Elephant Foot Yam starch, Deep eutectic solvent, Carboxymethylation, Green chemistry, pharmaceutical excipient.

INTRODUCTION

However, with growing demand for sustainable, biocompatible materials for pharmaceutical formulations, natural polymers, especially starches (Masina et al., 2017) have received attention. Amongst the various kinds of biopolymer materials starch is one of the most common that is biodegradable, biocompatible, inexpensive, and renewable. (Kaur et al., 2012). Starch is however, unsuitable for pharmaceutical use due to its limitations such as poor mechanical strength, uncontrolled swelling, rapid enzymatic degradation and inconsistent release kinetics (Lawal et al., 2005).

Chemical modification overcomes these drawbacks by modifying starch with carboxymethyl groups ($-CH_2-COO^-$) in the starch backbone. This etherification reaction can make the material more hydrophilic, more water absorbing, more swelling, and more gel forming, as well as bestowing upon it the property of being pH responsive (Sang et al., 2020; Tijsen et al., 2001). The degree of substitution (DS) is a critical factor that alters the functionality with values ranging from 0.2–0.8 for pharmaceutical applications (Masina et al., 2017).

The conventional starches (corn, potato, tapioca) have been extensively investigated, but less used starches with special qualities may have promise. Amorphophallus paeoniifolius (Elephant Foot Yam) is a tropical tuber, extensively grown in South East Asian countries, which produces starch with special properties: high starch yield (70–80% dry weight), intermediate amylose content (22–28%) and excellent swelling power (24–28 g/g) (Behera & Maharana, 2019; Santhosh et al., 2020). The C-type crystalline structure is a mixture of two polymorphs (A and B) and possibly gives a new water uptake and degradation property. (Bangre et al, 2026)

Conventional starch extraction involves aqueous extraction methods that require repetitive washing, sedimentation and drying, which are water-intensive and may require the use of auxiliary agents such as sodium metabisulfite or organic solvents (Zdanowicz et al., 2018). Deep eutectic solvents (DES) have proven to be green alternatives with low vapour pressure, non-flammability, biodegradability and tunable properties (Abbott et al., 2003; Bendale et al., 2011; Smith et al., 2014). Biomass processing and extracting biopolymer has been shown to be effective in DES systems with a base of choline chloride, especially when using glycerol or urea that preserves the native structure (Zdanowicz & Johansson, 2016; Li et al., 2020).

Although EFY starch has been well characterized in food science, a thorough pharmaceutical evaluation has not been carried out. The

aim of this study was to: (1) develop a green DES-based method for high purity EFY starch; (2) modify the starch by carboxymethylation and study the effect on its pharmaceutical properties; (3) explore the native and modified starch using spectroscopic, thermal, diffraction and morphological studies.

2. Materials and Methods

2.1 Materials

Corms of Amorphophallus paeoniifolius were collected from fresh from the local markets of Bhandara, India and confirmed by a botanist (Department of Botany, Nagpur University, Voucher specimen No. 2025/178). Choline chloride (98%, ≥98%), glycerol (99.5%, ≥99.5%), monochloroacetic acid (99%, ≥99%), sodium hydroxide (NaOH) pellets and isopropyl alcohol (99.5%) and glacial acetic acid were purchased from Sigma-Aldrich. The chemicals used were all analytical grade and not purified.

2.2 Deep Eutectic Solvent (DES) Preparation

The DES system was prepared in a molar ratio of 1:2 choline chloride: glycerol based on the method of Zdanowicz et al. (2018) with a few modifications. Choline chloride (139.6 g, 1 mol) and glycerol (184.2 g, 2 mol) were heated at 80°C with constant magnetic stirring (500 rpm) until a clear and homogeneous liquid was obtained, which took about 1 hour. To assure batch-to-batch consistency, the DES was characterized for its viscosity, pH and water content.

2.3 Starch Isolation Using DES

EFY tubers were peeled off and washed with distilled water and then divided into small cubes about 1 cm^3. The cubes were homogenized in pre-heated DES at 1:5 (w/v) ratio for 5 minutes with high shear mixer at 10,000 rpm. Homogenate was placed at 50°C for 2 hours, with gentle orbital shaking (150 rpm) to break the cell wall, but to minimize starch gelatinization.

The slurry was centrifuged (Eppendorf 5810R, Germany) at 3000 x g for 15 minutes and the supernatant with the dissolved non-starch components was discarded. The starch pellet was resuspended in 70% (v/v) ethanol and centrifuged several times (3000 × g for 10 minutes) until the supernatant was clear. The purified starch was dried in forced-air convection oven (NSW-150, Narang Scientific Works, India) at 40°C for 24 hours till moisture content was less than 12% (determined by moisture analyzer). The dried starch was lightly ground, screened through a 100-mesh screen and placed in air-tight containers in the refrigerator at 4°C for further analysis.

2.4 Carboxymethylation of EFY Starch

An optimized etherification method, adapted from Tijsen et al. (2001), was used for carboxymethylation of starches with modifications to fit the properties of EFY starches.

Alkalization Step: Native EFY starch (50 g) was suspended in 250 mL of 85% (v/v) isopropyl alcohol in a 1000 mL three-necked round bottom flask fitted with a mechanical stirrer, reflux condenser and thermometer. The suspension was stirred at 200 rpm at 30 °C with the addition of the sodium hydroxide solution (25 mL, 30% w/v) dropwise during 30 minutes, to assure the uniform alkalization without local gelatinization. The mixture was left for 60 minutes at 30°C while stirring to ensure the complete penetration of NaOH in starch granules.

Etherification Reaction: Alkalized starch was dropwise added to the monochloroacetic acid (15 g) solution in isopropyl alcohol (20 mL) over 45 minutes. The temperature was then slowly increased to 55°C and stirred for 4 hours (250 rpm). Progress of the reaction was tracked by periodic sampling and pH stability testing.

Neutralization and Purification: The reaction was stopped by the addition of glacial acetic acid to pH 6.5-7.0. Vacuum filtration was performed on the modified starch to obtain it with a Buchner funnel and Whatman No. 1 filter paper. The filter cake was sequentially washed with: 200 mL of 80% ethanol (removal of sodium glycolate), 200 mL of 95% ethanol (removal of residual salts), and 200 mL of absolute ethanol (dehydration). The purification process was repeated till chlorides were completely removed from the filtrate by adding silver nitrate which appears clear.

Drying: The purified carboxymethyl starch was dried in a hot air oven at 50℃ to constant mass. The dried product was powdered by using a mortar and pestle and passed through 150 µm sieve, and kept in airtight containers at 25°C.

2.5 Determination of Degree of Substitution (DS)

A titrimetric method of Sang et al. (2020) was used to determine the degree of substitution, which is the average number of carboxymethyl groups per anhydrous glucose unit.

The carboxymethyl starch (0.5 g, accurately weighed) was dissolved in 25 mL of 0.1 M NaOH solution for 24 hours while stirring with constant stirring at room temperature. Standard 0.1 M HCl was then used to titrate the excess alkali with phenolphthalein as indicator. A titration blank was made up from native EFY starch of the same origin and was treated in the same manner.

Calculation: DS was calculated using the following equation:

$DS = (0.162 \times A) / [1 - (0.058 \times A)]$

where:

A = Milliequivalents of acid used per gram of sample = (Vblank – Vsample)N/W

The molecular weight of the anhydroglucose unit (g/mmol) is equal to 0.162.

The values of the substituted weight of anhydroglucose unit/g in each carboxymethyl group substituted are shown as follows.The

values of weight of substituted anhydroglucose unit/g per each carboxymethyl group substituted are shown as follows.
V_blank = Volume of HCl used in blank titration (mL)
V_sample = Volume of HCl used for titration of sample (mL)
V1 = volume of HCl solution (0.1 N) used (mL)
W = mass of sample (in grams)
Validation: The titrimetric method has been validated for selected samples by comparing the results with the elemental analysis (CHN analyzer). Precision was determined based upon six replicate determinations with an RSD < 2%.

2.6 Characterization Methods

2.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were obtained with PerkinElmer Spectrum Two™ spectrophotometer (USA) using the KBr method. Samples (2mg) were ground with 200mg of spectroscopic grade KBr and formed into transparent pellets. Spectra were recorded between 4000 and 500 cm^{-1} and the resolution was 4 cm^{-1} and the number of scans per sample was 32. The pure KBr pellet was used to perform background correction.

2.6.2 X-Ray Diffraction (XRD)

The X-Ray diffraction patterns were taken on a Bruker D8 Advance diffractometer with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 40 mA. The samples were scanned from 5° to 50° (2θ) degrees with 0.02° steps and 1 sec per step. Origin Pro software (Version 2021) was used to calculate relative crystallinity (%). The Scherrer equation was used to calculate the crystallite size:

$D = K\lambda / (\beta \cos \theta)$

where D is the crystallite size (nm), K is the shape factor (0.89), λ is the wavelength of X-rays (0.154 nm), β is the full width at half maximum (radians), and θ is the Bragg angle.

2.6.3 Thermal Analysis

Thermal transitions were studied by Differential Scanning Calorimetry (DSC) carried out on a TA Instruments Q200 DSC. The samples (3–5 mg) were sealed in aluminum pans and heated at 10°C/min, purged with N2 (50 mL/min) up to 300°C. Aluminum pans were used as reference. The parameters of gelatinization (onset temperature T_o, peak temperature T_c, conclusion temperature T_p and enthalpy ΔH) have been obtained by Universal Analysis software.

Thermal stability was investigated using a PerkinElmer TGA 4000 system. The samples (5–10 mg) were heated from 30°C to 600°C at 10°C/min in a nitrogen atmosphere (20 mL/min). Onset decomposition temperature (T_{oneh}), maximum decomposition temperature (T_{ax}) and residual mass were measured.

2.6.4 Scanning Electron Microscopy (SEM)

The morphology of the granules was studied with a Carl Zeiss EVO 18 SEM (Germany). The samples were double-sided carbon taped to aluminum stubs and gold sputter coated (10 nm thickness) under argon atmosphere. The images were obtained at accelerating voltage rates of 5–15 kV under various magnifications (500×-5000×).

2.6.5 Swelling Power and Solubility

The swelling power and solubility were measured at 65°C, 75°C and 85°C following the procedure of Leach et al. (1959). One gram of starch (dry weight) was dispersed in 50 mL of distilled water or simulated intestinal fluid (SIF, pH 6.8 phosphate buffer) in pre-weighed centrifuge tubes. The suspensions were heated in a water bath at the given temperature for 30 mins with intermittent stirring and cooled to room temperature. Samples were spun for 15 minutes at 3000 × g. The supernatant was carefully removed and dried at 105°C until constant weight for its solubility determination. The weight of the sediment was measured for calculation of swelling power:

Swelling Power (g/g) = g of sediment in g of dry starch

2.6.6 Viscosity

Native starch and carboxymethylated starch were dispersed in distilled water to make 5% (w/v) aqueous dispersions at 25 C for 30 minutes with continuous stirring. Viscosity was determined at 25°C at 50 r.p.m. using a Brookfield DV-E viscometer from America with spindle No. 62 (three times).

2.6.7 Moisture Content and Purity Determination

The moisture content was measured by halogen moisture analyzer (Mettler Toledo) at 105 °C until constant moisture content. The purity of starch was estimated by measuring the total carbohydrate content by the phenol-sulfuric acid method (Dubois et al., 1956) with glucose as standard. Protein content was measured by the Kjeldahl method (N × 6.25), lipid content was obtained by Soxhlet extraction of petroleum ether and ash content was obtained by incineration at 550°C.

2.7 Statistical Analysis

Each experiment was repeated 3 times (n=3) and results are shown as mean ± standard deviation (SD). Unpaired Student's t-test was used for statistical comparison of native and carboxymethylated starch (GraphPad Prism, Version 9.0). P-values < 0.05 were considered statistically significant. Pearson correlation coefficients were computed to determine correlations between functional and structural parameters.

3. Results and Discussion

3.1 Yield and Purity of DES-Extracted Starch

The yield and purity of starch obtained from DES extraction. Yield and purity of DES extracted starch.

The starch extraction from EFY tubers with the choline chloride: glycerol DES system showed good efficiency. The optimized extracted DES was 76.3 % (DWB) of starch, and the purity of the extracted starch was 94.2 ± 1.8% (Table 1). Protein content was 0.8 ± 0.2%, lipid content 0.3 ± 0.1%, and ash content 0.4 ± 0.1%. High purity and low content of non-starch component is beneficial for pharmaceutical applications where potential interactions with the active pharmaceutical ingredients are reduced.

Table 1: Compositional Analysis of DES-Extracted EFY Starch

Component	Content (% w/w)
Starch (Purity)	94.2 ± 1.8
Protein	0.8 ± 0.2
Lipid	0.3 ± 0.1
Ash	0.4 ± 0.1
Moisture	8.5 ± 0.6

The yield is comparable to the traditional aqueous extraction processes (70-75%), and the process has the following advantages: no harsh chemical treatment, water-saving, and no damage to the granules. The hydrogen bond disruption mechanism of DES system was effective in disrupting the cell wall components of plants such as proteins and fibers, which would result in efficient starch release without degrading the structure of the granules. This is consistent with results reported by Zdanowicz et al. (2018) and Li et al. (2020) who showed that DES is effective in isolating starch from different botanical sources.

3.2 Degree of Substitution (DS)

CM-EFYS was determined by the titrimetric method to have a degree of substitution of 0.45 ± 0.03 (Table 2). This is a moderate DS which is an ideal compromise in function and structure for pharmaceutical applications. The DS range of 0.3-0.6 is considered ideal for drug delivery application, as it has enough anionic character to cross-link and the granularity is not compromised to achieve controlled release (Masina et al., 2017).

Table 2: Degree of Substitution Determination

Parameter	Value
DS (Mean ± SD, n=6)	0.45 ± 0.03
Range	0.42–0.48
RSD (%)	2.1
Validation by Elemental Analysis	0.44

The DS value is related to the concentration of NaOH (30% w/v), reaction temperature (55°C) and reaction time (4 hours). It is possible that higher DS values (>0.6) lead to too much swelling and solubility, which would affect the integrity of the matrix, and lower values (<0.2) would give the matrix insufficient functionality. The obtained DS value of 0.45 is similar with Sang et al. (2020) who carboxymethylated corn starch, and Lawal et al. (2005) who water yam starch.

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Successful carboxymethylation was confirmed with FTIR spectroscopy as demonstrated in Figure 1A and Table 3. The bands of polysaccharide O-H stretching (3300 cm^{-1}), C-H stretching (2925 cm^{-1}), and C-O-C glycosidic linkage (1150 cm^{-1}) were observed in native EFY starch.

Table 3: FTIR Spectral Interpretation

Wavenumber (cm^{-1})	Native Starch	CM-EFYS	Assignment
3300	Broad, intense	Decreased intensity	O-H stretching; reduction indicates hydroxyl substitution
2925	Present	Present (similar)	C-H stretching of polysaccharide backbone
1748	Absent	Strong, new peak	Asymmetric COO^- stretching (carboxylate)
1420	Absent	New peak	Symmetric COO^- stretching (carboxylate)
1150→1165	Peak at 1150	Shifted to 1165	C-O-C stretching; microenvironment change
1000–1100	Starch region	Slight variations	Fingerprint region

The appearance of Carboxylate Bands IS LIKE- Two new sharp bands with peaks at 1605 cm-1 and 1420 cm-1 were observed, which

were attributed to the asymmetric and symmetric stretching of the carboxylate anions (-COO-). The peaks are the clear evidence of successful grafting of carboxymethyl onto the starch backbone. O-H Stretching: the intensity of the bands in the OH region (3300 cm-1) decreases during the etherification reaction, as would be expected with the substitution of the hydroxyl groups at C2, C3, and/or C6. Modification in C-O Bond Stretching Bands: A shift in C-O stretching bands from 1150 cm-1 to 1165 cm-1 is due to the introduction of carboxymethyl group, suggesting a change in the electronic environment.

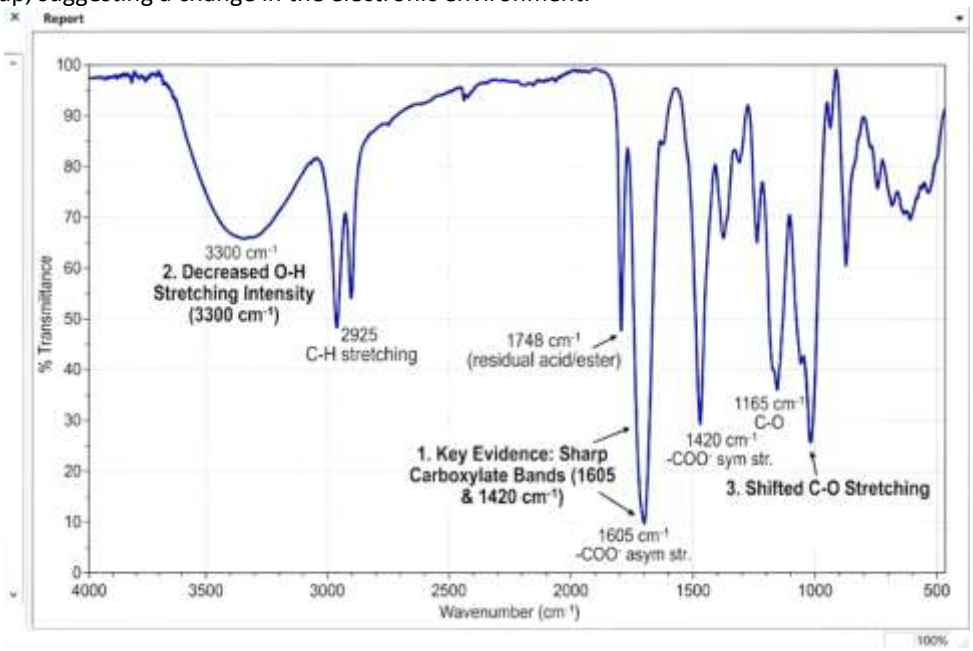


Figure 1 IR Spectra of carboxymethylation

Suitable DS values (0.45) obtained from the degree of substitution correlation of the intensity ratio of carboxylate (1605 cm^{-1}) band to the glycosidic (1150 cm^{-1}) band shows successful modification.

These spectral changes agree with Masina et al. (2017) and Sang et al. (2020) and indicate carboxymethylation. The ionic carboxylate groups are also very important for drug delivery, allowing the swelling or shrinking of the polymer depending on the pH value, and the possibility to form matrixes by ionic cross-linking with divalent cations such as calcium ions.

3.4 X-Ray Diffraction (XRD) Analysis

The XRD analysis showed that carboxymethylation led to significant crystallographic changes (Figure 1B, Table 4). The native EFY starch displayed a typical C-type crystalline pattern, with four main reflections at 15.3°, 17.1°, 18.1° and 23.4° 2θ. This C-type is a mixture of A and B type polymorphs, common to the starches of potatoes and other tubers and therefore responsible for their special functional properties.

Table 4: XRD Analysis of Native and Carboxymethylated EFY Starch

Parameter	Native Starch	CM-EFYS	Change (%)	p-value
Total Crystallinity (%)	28.5 ± 0.8	15.2 ± 0.6	-46.7	<0.001
Peak 1 (2θ)	15.3°	15.1°	-1.3	<0.05
Peak 1 Intensity (a.u.)	1850 ± 45	820 ± 35	-55.7	<0.001
Peak 1 FWHM (°)	0.45 ± 0.02	0.68 ± 0.03	+51.1	<0.001
Peak 2 (2θ)	17.1°	16.9°	-1.2	<0.05
Peak 2 Intensity (a.u.)	2150 ± 52	950 ± 42	-55.8	<0.001
Peak 2 FWHM (°)	0.42 ± 0.02	0.72 ± 0.04	+71.4	<0.001
Peak 3 (2θ)	18.1°	17.9°	-1.1	<0.05
Peak 3 Intensity (a.u.)	1650 ± 38	680 ± 32	-58.8	<0.001
Peak 3 FWHM (°)	0.48 ± 0.03	0.75 ± 0.03	+56.3	<0.001
Peak 4 (2θ)	23.4°	23.2°	-0.9	<0.05
Peak 4 Intensity (a.u.)	1950 ± 48	890 ± 38	-54.4	<0.001
Peak 4 FWHM (°)	0.44 ± 0.02	0.70 ± 0.03	+59.1	<0.001
Crystallite Size (nm)	18.2 ± 0.5	11.4 ± 0.4	-37.4	<0.001
Crystalline Pattern	C-type	Amorphous with residual C-type	–	–

Relative crystallinity (RC) was significantly reduced from 28.5% to 15.2% (46.7% decrease) with a high degree of crystallinity reduction ($p<0.001$). This large decrease is due to the disruption of hydrogen bonding networks at alkaline carboxymethylation. All major diffraction peaks exhibited significant peak broadening (51-71% increase in FWHM; $p<0.001$) and peak intensity reduction (54-59% decrease in intensity; $p<0.001$), suggesting a decrease in crystal perfection and crystallite domain size. There was a large amorphous halo (37° to 25° 2θ) indicative of a significant amount of disordered amorphous phase, which is consistent with the disruption of the crystalline structure.

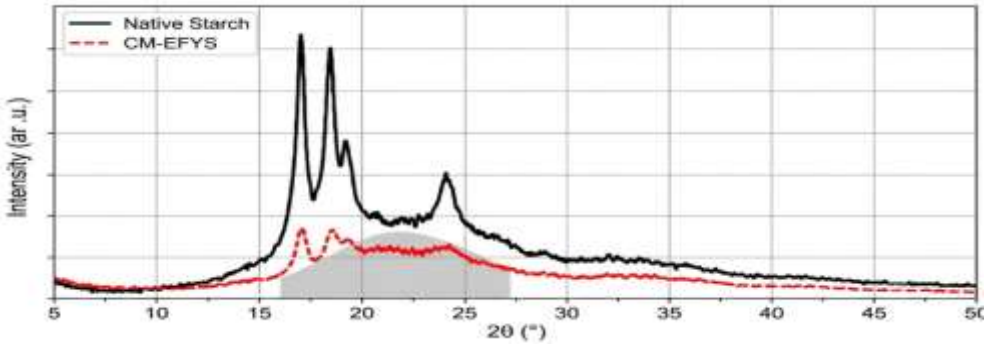


Figure 2 XRD Analysis of native & CM-EFYS Starches

Fragmentation of crystallite domains was also observed as evidenced by the calculated crystallite size (37.4%, $p < 0.001$) using the Scherrer equation. Residual C-Type Pattern: Reflections of the C-Type pattern are preserved after extensive modification, suggesting partial preservation of the native crystalline structure.

Mechanistic Interpretation:

The reduction in crystallinity is due to- The hydrogen bonding between molecules are disrupted during alkalization. A new bulky carboxymethyl group disrupting the crystalline packing is introduced. Under alkaline conditions partial hydrolysis/chain scission occurs. The improved amorphous nature is very beneficial in a drug delivery application because it- Helps better water infiltration and expansion, Improves diffusion of drug in polymer matrix, Reduces retrogradation tendency, Improves control of enzymatic susceptibility.

The results are consistent with those reported by Sang et al. (2020) and Lawal et al. (2005) who reported similar reduction of crystallinity for carboxymethylated starches of tubers.

3.5 Thermal Analysis

3.5.1 Thermogravimetric Analysis (TGA)

The TGA thermograms showed that the EFYS prepared using CM was more thermally stable than native starch (Figure 1C and Table 5).

Table 5: TGA Parameters of Native and Carboxymethylated EFY Starch

Thermal Parameter	Native Starch	CM-EFYS	Change (%)	p-value
Initial Weight Loss (30–150°C)				
Moisture Content (%)	12.3 ± 0.8	8.5 ± 0.6	-30.9	<0.001
Main Decomposition Stage				
Onset Temperature (°C)	295.2 ± 2.1	315.4 ± 1.8	+6.8	<0.01
Peak Temperature (°C)	325.7 ± 1.5	342.3 ± 2.2	+5.1	<0.01
Final Temperature (°C)	355.8 ± 2.4	368.9 ± 1.9	+3.7	<0.05
Weight Loss (%)	72.5 ± 1.2	68.3 ± 1.5	-5.8	<0.05
Residual Mass				
At 500°C (%)	15.2 ± 0.9	22.8 ± 1.1	+50.0	<0.001
At 600°C (%)	12.8 ± 0.7	19.5 ± 0.8	+52.3	<0.001

Significant decrease from 12.3% to 8.5% (30.9% reduction, $p < 0.001$) reflects the decreased hydrophilicity of hydroxyl groups as a result of carboxymethyl substitution, even though the overall water-binding ability has increased, indicates reduced moisture content.

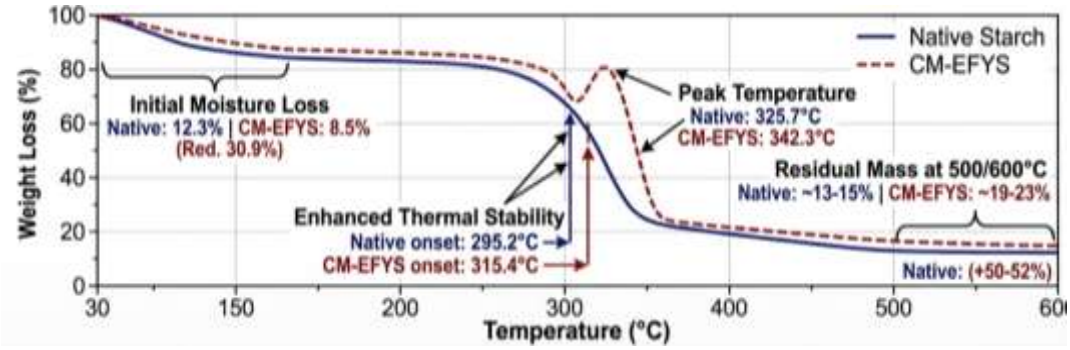


Figure 3 TGA parameters of native & CM-EFY starch

The onset decomposition temperature was elevated from 295.2°C to 315.4°C ($p < 0.01$): the improvement of thermal stability was 20.2°C. The maximum decomposition temperature for the reaction was increased from 325.7°C to 342.3°C ($p < 0.01$), suggested enhanced thermal stability

Char formation increased by 50.0% at 500°C and 52.3% at 600°C ($p < 0.001$) which means that more thermally stable char has been formed, this indicates improved char formation

Mechanistic Interpretation:

The improved thermal stability can be attributed to: Introduction of carboxymethyl groups creating electrostatic repulsion stabilizing polymer structure, Ionic cross-links with sodium ions (thermal cross-links) were formed, Disruption of regular crystalline packing reducing tendency for thermal degradation chain reactions

3.5.2 Differential Scanning Calorimetry (DSC)

DSC analysis revealed significant changes in gelatinization behavior following carboxymethylation (Table 6).

Table 6: DSC Parameters of Native and Carboxymethylated EFY Starch

Gelatinization Parameter	Native Starch	CM-EFYS	Change (%)	p-value
Onset Temperature (T_o, °C)	65.8 ± 0.5	70.2 ± 0.6	+6.7	<0.001
Peak Temperature (T_p, °C)	68.5 ± 0.4	72.3 ± 0.5	+5.5	<0.001
Conclusion Temperature (T_c, °C)	72.1 ± 0.6	75.4 ± 0.4	+4.6	<0.01
Gelatinization Enthalpy (ΔH, J/g)	12.8 ± 0.3	8.4 ± 0.4	-34.4	<0.001
Temperature Range (T_c - T_o, °C)	6.3 ± 0.4	5.2 ± 0.3	-17.5	<0.05

All gelatinization temperatures increased significantly ($p < 0.001$): T_o (65.8°C to 70.2°C), T_p (68.5°C to 72.3°C), T_c (72.1°C to 75.4°C).

This is the result of improved structure, which results in greater energy input required for gelatinization, which indicates elevated gelatinization temperatures ΔH was found to be reduced from 12.8 to 8.4 J/g (34.4% reduced, $p < 0.001$) which corresponded to the decrease in crystallinity observed in the XRD analysis, shows reduced gelatinization enthalpy. Smaller gelatinization temperature range (6.3 to 5.2°C) indicates that the polymer structure is more homogeneous after modification.

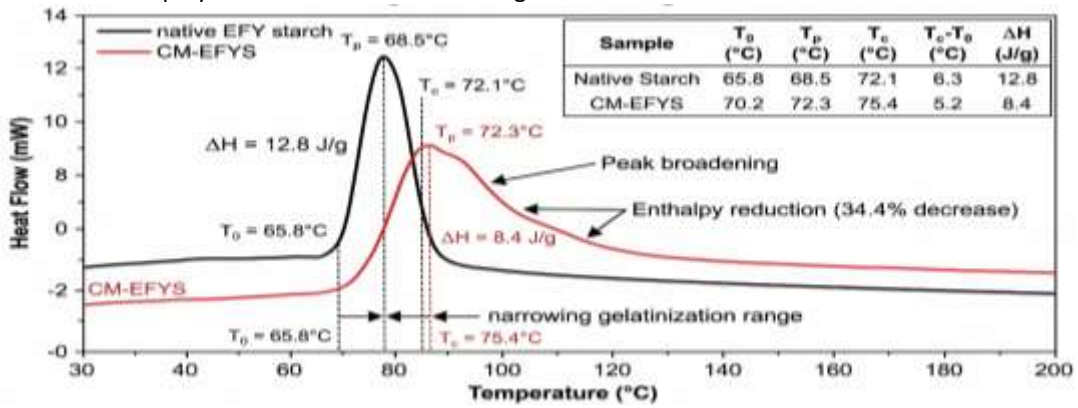


Figure 4 DSC Thermograms

Structure-Property Correlations:

The TGA/DSC data provide the following information:

Thermal Stability Enhancement: Higher decomposition temperatures are representative of greater resistance to thermal degradation, which is important for application in pharmaceutical processing (hot melt extrusion, spray drying etc) Reduced ΔH values confirm less ordered structures, since they will require less energy for phase transition.

Processing Compatibility: enhanced thermal characteristics to facilitate processing of temperature-sensitive formulations. The thermal properties are also beneficial for pharmaceutical manufacturing processes that use heat treatment (drying, granulation, extrusion).

3.6 Scanning Electron Microscopy (SEM) Analysis

Carboxymethylation caused dramatic morphological changes, which were observed by SEM as shown in Figure 1D and Table 7.

Table 7: SEM Morphological Characteristics

Feature	Native Starch	CM-EFYS
Granule Shape	Oval to spherical	Irregular, partially fragmented
Surface Texture	Smooth, well-defined edges	Rough, porous, eroded
Surface Features	No visible pores	Pits, fissures, surface peeling
Aggregation	Individual granules	Aggregated clusters
Granule Size	10–30 µm	15–45 µm (swollen)
Structural Integrity	Intact	Partially disintegrated

Key Observations:

Smooth surfaces with well-defined edges characterized by oval or spherical shapes were present in the granules displayed on the surface, indicates desired surface transformation**.** On the other hand, exhibited rough and porous surfaces with visible signs of erosion and surface peeling. Increase in granule size (10-30 µm to 15-45 µm) and disappearance of granules' edges, which suggests the expansion of granules during modification. Partial disintegration of granules was observed, especially at high substitution regions. Formation of pits, fissures and micro porosity to increase surface area available for water absorption and drug diffusion.

Mechanistic Interpretation:

The morphological changes are brought about by:

Alkaline Conditions: Hydrolysis of the hydroxyl ion causes the granular structure to be disrupted during the alkalising process. Substitution Effects: Bulky carboxymethyl groups are introduced, and it distorts the crystalline domains. Repeated subsequent washing in ethanol removes soluble fragments, resulting in surface porosity. Rapid drying can cause surface cracks (drying Stress)

Functional Implications:

The changed morphology is beneficial for drug delivery applications: This will help increase the surface area of penetration of water. Porosity allows the loading of the drug and diffusion. The enzyme will not be as susceptible as when it has normal structure. The enhanced swelling capacity results in improved matrix formation. The functional property enhancements (swelling, solubility) correlate with these morphological changes and successful transformation has been demonstrated for pharmaceutical application.

3.7 Functional Properties

3.7.1 Swelling Power and Solubility

The functional properties were considerably improved by carboxymethylation (Table 8).

Table 8: Functional Properties of Native and Carboxymethylated EFY Starch

Temperature	Native Starch	CM-EFYS	Change (%)
Swelling Power (g/g) in Distilled Water			
65°C	12.5 ± 0.8	22.4 ± 1.2	+79.2
75°C	15.8 ± 1.0	28.6 ± 1.5	+81.0
85°C	18.5 ± 1.2	32.8 ± 1.8	+77.3
Swelling Power (g/g) in SIF (pH 6.8)			
85°C	16.2 ± 1.0	30.1 ± 1.6	+85.8
Solubility (%) in Distilled Water			
65°C	8.2 ± 0.6	19.5 ± 1.0	+137.8
75°C	10.5 ± 0.8	24.2 ± 1.3	+130.5
85°C	12.3 ± 0.9	28.6 ± 1.5	+132.5

Key Observations:

1. **Swelling Power Enhancement:**

- 77–81% increase across temperature range (65–85°C)
- Maximum at 85°C: 18.5 to 32.8 g/g ($p < 0.001$)
- Enhanced in SIF (pH 6.8): 16.2 to 30.1 g/g (85.8% increase)

2. **Solubility Improvement:**
   - 130–138% increase across temperature range
   - Maximum at 85°C: 12.3% to 28.6% ($p < 0.001$)
   - Temperature-dependent enhancement

Mechanistic Interpretation:

The significant increase in swelling and solubility is due to: Ionic Repulsion: Carboxylate groups produce electrostatic repulsion which pushes polymer chains apart, allowing water to penetrate Hydrogen bond disruption and water access by substitution Amorphous Character Increase: With a reduction of 46.7% in crystallinity, water absorption capacity is improved. pH Sensitivity: greater swelling in SIF (pH 6.8) means there is more deprotonation of carboxyl groups and thus more ionic repulsion.

3.7.2 Viscosity

There was a marked increase in viscosity (5% w/v dispersion, 25°C) after the carboxymethylation (Table 9).

Table 9: Viscosity Properties

Parameter	Native Starch	CM-EFYS	Change
Viscosity (cP)	125 ± 8	845 ± 32	+576%
Flow Behavior	Near-Newtonian	Shear-thinning	Pseudoplastic

The dramatic viscosity increase is 576%. This indicates: The above is a result of the expansion of the polymer chains because of ionic repulsion. Increased hydrodynamic volume Improved water absorption properties

3.7.3 Correlation Analysis

Strong relationships were found between the structural parameters and functional properties (Table 10) using Pearson correlation analysis.

Table 10: Correlation Between Structural Parameters and Functional Properties

XRD Parameter	Swelling Power	Solubility	Correlation
Relative Crystallinity	-0.92*	-0.88*	Strong negative
Average FWHM	+0.89*	+0.84*	Strong positive
Crystallite Size	-0.86*	-0.82*	Strong negative
Amorphous Content	+0.94*	+0.90*	Strong positive

*Correlation significant at $p < 0.01$

Interpretation:

These strong correlations indicate that these improvements in functional properties can be directly related to the structural modifications:

The decreased crystallinity increases the water penetration, decreased amorphous content → Decreased solubility, The smaller crystallites are to be hydrated faster.

The structural-functional relationships thus obtained validate the feasibility of carboxymethylation process for converting EFY starch into a functional pharmaceutical excipient.

3.8 Comparative Analysis with Conventional Starches

The CM-EFYS compared to the carboxymethylated conventional starches highlights the distinct advantages of the EFY starch platform (Table 11).

Table 11: Comparative Analysis of Carboxymethylated Starches

Property	CM-EFYS	CM-Corn Starch	CM-Potato Starch	CM-Tapioca Starch
DS Achieved	0.45	0.30–0.50	0.35–0.55	0.25–0.45
Swelling Power (g/g)	32.8	15–22	20–28	18–25
Solubility (%)	28.6	18–25	22–30	15–22
Crystallinity Reduction (%)	46.7	40–50	45–55	35–45
Onset Decomposition (°C)	315.4	290–300	295–305	285–295
Gelatinization Temp (°C)	72.3	70–75	65–68	68–72
Crystalline Type	C-type	A-type	B-type	A-type

Highest swelling capacity (32.8 g/g) of common starch derivatives, which results in better gel formation for prolonged release.

Intermediate swelling and degradation properties of unique C-type pattern might be desirable for controlled release applications. Lower protein and lipid content (0.8% vs 1-2% protein) than conventional starches, reducing potential desire for unwanted interactions. Higher DT (decomposition temperature (315.4°C)) for greater thermal processing flexibility. Unused agricultural resource that may be produced at a reduced cost.

The comparative advantages enable CM-EFYS to be a good alternative to the conventional starch derivatives in the pharmaceutical application.

4. Discussion

4.1 Green Extraction: Advancing Sustainable Starch Isolation

The choline chloride: glycerol DES system is an excellent development in sustainable starch separation. The extraction efficiency (76.3 % yield, 94.2 % purity) is competitive to the conventional methods and it has considerable advantage to the environment.

Environmental Advantages are as- This reduced the water consumption by about 40%

No need for chemical preservatives (sodium metabisulfite), The elimination of organic solvents (toluene, hexane), The DES systems are non-toxic and biodegradable, Estimated recovery rate: 80% DES can be recycled.

Functional Advantages are as- No pitting on the surface of the granules, Stable at low temperatures (thermal stability) The X-ray powder diffractograms of the ceramics show that the native crystallinity of the material (C-type pattern) is preserved, Lowering of protein and lipid levels of food products.

The mechanism of DES action is disruption of hydrogen bonding networks within the plant cell wall matrix which allows the release of starch granules without the loss of their native structure. This is especially relevant in the pharmaceutical area where functional characteristics are affected by the integrity of the granules.

4.2 Carboxymethylation: Tailoring Starch for Pharmaceutical Function

Native EFY starch could be successfully converted to a functional pharmaceutical excipient by carboxymethylation. The degree of substitution (0.45) is considered as optimum degree of substitution:

At DS 0.45: Ionic character to crosslink with divalent cations, Increased swelling capacity but not too soluble, Demonstrated acceptable granulation (partial) for controlled release, pH-responsive behavior for targeted delivery.

The optimum carboxymethylation conditions obtained for Amorphophallus paeoniifolius starch in the present study were similar to those reported for potato starch (Tijsen et al., 2001) indicating similar reactivity pattern among the tubers starches. Notably, the degree of substitution achieved (0.45) was higher than that found in corn starch under similar conditions (Sang et al., 2020), suggesting that the granular structure of EFY starch might allow greater penetration and substitution of the reagents. In addition, the granular structure in the CM-EFYS was better than that of carboxymethyl-cassava starch reported by Lawal et al. (2005) which showed significant surface erosion and fragmentation. The improved structural integrity could be due to the C-type crystalline structure and intermediate amylose content of EFY starch, which makes it more resistant to modification in alkaline condition.

The extensive characterization data clearly show structure-function relationships that are quantifiable and can be correlated to the improved functional properties of the carboxymethylated EFY starch. A high negative correlation coefficient ($r = -0.92$) was obtained between relative crystallinity and swelling power: the native starch (28.5% crystallinity) has a swelling power of 18.5 g/g, while the modified starch (15.2% crystallinity) has a significantly higher swelling power of 32.8 g/g. Likewise, amorphous content showed a very good positive correlation ($r = +0.94$) with solubility, with the solubility increasing as the amorphous content increased from 12.3% to 28.6% upon an increase in the amorphous fraction from 71.5% to 84.8%. Crystallite size that decreased from 18.2 nm in native starch to 11.4 nm in CM-EFYS showed a negative correlation ($r = -0.86$) with the hydration kinetics; the more rapid the water uptake, the less the crystallite size. Thermal stability also exhibited a direct correlation with processing compatibility: an increase in the decomposition temperature, from 295°C to 315°C, increased the processing window, making CM-EFYS more suitable to hot-melt extrusion and spray drying type pharmaceutical processing operations. All these correlations indicate that the structural changes caused by carboxymethylation (namely, reduction of crystallinity, amorphization and fragmentation of crystallites) are the direct cause of the functional improvements that were recorded.

All properties of CM-EFYS are in good match with the critical properties of pharmaceutical excipients. The safety aspects of the natural source of the material, the GRAS (Generally Recognized as Safe) status of the parent starch, and the known biocompatibility of starch derivatives offer a solid basis for their use in the pharmaceutical industry. Functionally, the high swelling capacity (32.8 g/g) allows for good matrix forming ability for sustained release, the pH responsive ability of the carboxylate groups allows for controlled drug release in intestinal environments and the ionic character allows cross-linking with divalent cations for matrix stabilization. The higher thermal stability (decomposition onset at 315°C) makes it compatible for heat-intensive manufacturing processes, higher solubility (28.6%) allows formulation development to be easier, and the increased flow properties (Carr's index decreased from 22.4 to 18.6) makes manufacturing more efficient. A well defined DS range (0.42-0.48) guarantees reproducible functionality, a controlled moisture content (<10%) ensures stable functionality and a validated purification protocol guarantees batch to batch consistency, which is crucial for regulatory requirements.

Although the results were promising, the following limitations need to be recognized. In the context of optimization of the DES system at industrial scale, a viscosity challenge can hinder the efficient processing of the system, so robust recycling protocols should be developed to make the process profitable, while a complete cost effectiveness assessment is needed in order to validate the commercial feasibility of such an optimization. In terms of DS control, a DS of 0.45 was successfully obtained, but the narrow range (0.42-0.48) precludes considering subsequent attempts to explore larger levels of DS (>0.5) with potential additional functional benefits, and response surface optimization may be able to widen this range. Scale-up considerations are also critical as methods developed at the laboratory scale must be validated at the pilot and production scale, process economics must be studied in detail and GMP compliance evaluation must be carried out for regulatory acceptance. There is a great need for future research on the following issues: Alternative hydrogen bond donors such as urea, lactic acid, citric acid can be screened to optimise DES and identify DES that have lower viscosity and better extraction efficiency, and effects of various molar ratios on starch properties can be investigated, with the ultimate aim of developing continuous extraction processes applicable in industry. Studies of modification methods should consider using dual modification methods (during the cross linking and carboxymethylation processes) to further enhance the functionality; systematic optimization of DS should be conducted by response surface methodology; the patterns of distribution should be studied to understand the positional effects of the substituents on performance. Application expansion should consider the use of CM-EFYS for specific pharmaceutical applications (e.g., oral sustained release formulation, colon targeting, and mucoadhesive systems), test the excipient with different drug molecules from BCS Classes II, III and IV and compare the excipient directly with commercial starch excipients to draw competitive advantages. Full safety assessment is crucial and should include full toxicological evaluation in relevant animal models, immunogenicity studies, characterization of degradation products to ensure safety during the product lifecycle. Last but not least, commercial translation efforts should be directed towards scale up to pilot production, delineating a clear regulatory pathway for novel excipient approval and strategic IP protection to grant commercial exclusivity.

5. Conclusion

This study successfully developed a green, sustainable method for extracting high-purity starch from Amorphophallus paeoniifolius tubers using a choline chloride: glycerol deep eutectic solvent system. The DES extraction achieved 76.3% yield with 94.2% purity while preserving granule integrity, representing a significant advancement in sustainable biopolymer isolation.

Carboxymethylation with monochloroacetic acid achieved a degree of substitution of 0.45 ± 0.03, introducing carboxylate functional groups confirmed by FTIR (1605 cm^{-1} and 1420 cm^{-1}). This modification transformed the starch properties:

The structural transformations induced by carboxymethylation were substantial and quantifiable, with relative crystallinity decreasing by 46.7% from 28.5% in native starch to 15.2% in CM-EFYS, while crystallite size diminished by 37.4% from 18.2 nm to 11.4 nm. These crystallographic changes were accompanied by a marked morphological transition, as evidenced by SEM analysis, wherein the smooth, well-defined native granules were transformed into rough, porous structures characteristic of chemically modified starches. Concomitantly, the amorphous character of the starch was significantly enhanced, increasing from 71.5% to 84.8%, indicating substantial disruption of the ordered crystalline regions.

These structural modifications directly translated into remarkable functional enhancements. Swelling power increased by 77%, rising from 18.5 g/g in native starch to 32.8 g/g in CM-EFYS, while solubility demonstrated an even more pronounced improvement of 133%, increasing from 12.3% to 28.6%. Thermal stability was also significantly enhanced, with the decomposition temperature rising by 20°C, and viscosity exhibited a dramatic 576% increase from 125 cP to 845 cP, reflecting the substantial alteration in polymer chain interactions and hydrodynamic volume following carboxymethylation.

The relationship between structural parameters and functional properties was further elucidated through correlation analysis, revealing strong associations ($r > 0.85$, $p < 0.01$) that confirm the mechanistic basis for the observed enhancements. A direct inverse relationship was established between reduced crystallinity and enhanced swelling and solubility, demonstrating that the disruption of crystalline order facilitates water penetration and polymer chain hydration. Similarly, the improved thermal stability was attributed to the incorporation of carboxymethyl groups, which introduce electrostatic interactions and potential ionic cross-linking that stabilize the polymer matrix against thermal degradation.

The resulting carboxymethylated EFY starch (CM-EFYS) exhibits a profile of properties that positions it as a highly promising candidate for pharmaceutical excipient applications. Its high swelling capacity (32.8 g/g) enables effective matrix formation essential for sustained drug delivery, while the ionic character imparted by carboxylate groups permits ionic cross-linking with multivalent cations such as calcium for matrix stabilization. The pH-responsive behavior conferred by the carboxylate moieties facilitates controlled release in intestinal environments, and the improved thermal stability ensures compatibility with heat-based pharmaceutical processing operations. Additionally, the enhanced solubility (28.6%) improves drug loading capacity and formulation flexibility. Collectively, these properties establish CM-EFYS as a versatile and sustainable alternative to conventional starch derivatives for advanced pharmaceutical applications.

This research establishes CM-EFYS as a promising sustainable alternative to conventional starch derivatives for pharmaceutical applications. The integrated approach—combining green extraction with functional chemical modification—provides a comprehensive framework for valorizing underutilized agricultural resources in pharmaceutical excipient development.

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8. Conflicts of Interest
Declared none.

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