

## Research Article

# Study on the Effect of Etherification on Physicochemical, Pasting, Morphological and Thermal Properties of Buckwheat Starch (*Fagopyrum Esculentum*)

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## Abstract

Carboxyl methyl starch was prepared by etherification process of native buckwheat starch by monochloroacetic acid at different pH. Response surface methodology with central composite experimental design at two level and two-factor (pH and temperature) was used for experiments. Effect of time and pH was accessed and varied from 30 to 120 min and 7 to 12.7 respectively. Degree of substitution (DS) varied from 0.05 to 0.392 of modified starch were studied on various physiochemical, pasting, morphological and thermal properties of native and modified starch. Swelling power (SP), solubility (S) and water binding capacity (WBC) were increased whereas decrease in syneresis signifies the higher freeze thaw stability of modified starch. Peak, breakdown and setback viscosity were decreased upon etherification while trough and final viscosity were increased. Morphological studied reflects the smoother surface of native starch and after modification it changes its behavior from crystalline to amorphous. The modification of starch upon etherification significantly affected the starch properties which would be helpful for the development of modified starch products with desirable characteristics.

**Keywords:** Buckwheat, starch, Carboxylmethyl, etherification, physicochemical

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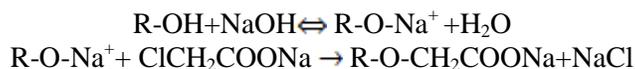
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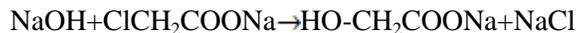
## Introduction

Starch is the most abundant storage carbohydrate in staple foods such as cereals, roots and tubers. Starch is a polysaccharide of glucose and widely used in different industries due to its low cost, biodegradability and renewability. Buckwheat comes under the categories of pseudo cereal which contain 65-70% of starch. But utilization of native starches in food formulations and industrial applications is limited due to some undesirable properties such as lack of free-flowing properties, insolubility in cold water, uncontrolled viscosity after cooking, low mechanical properties, and instability at high temperature, pH and shear during processing/cooking [1, 2]. Therefore, modifications of starch become important for the specific industrial processes. Modification occurs either by substituting the hydroxyl group in the starch chain or by breaking the chain into smaller fragments [3]. Thus, the alteration of structure at molecular level and affecting the hydrogen bonding in a controllable manner to enhance and extend their application. The modification caused by the chemical substitution is measured by the degree of substitution as the average value of substituted hydroxyl groups per glucose in starch chain [4].

## Reaction mechanism

Carboxymethyl starch (CMS) is obtained as a product of the reaction of starch and monochloroacetate in the presence of sodium hydroxide. This is a two-step reaction, the first step being the alkalization of starch [5]:





Where, R is the starch backbone.

The amount of carboxymethyl group formed is indicated by the degree of substitution and the most important methods include aqueous, dry and organic solvent method [6]. The objective of the present study was to investigate the effect of etherification on physicochemical, pasting, morphological and thermal properties of buckwheat starch.

## Materials and Methods

Native starch was isolated from buckwheat flour in Food Analytical Lab, Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, India.

### Sample preparation

Carboxymethyl starch of different degree of saturation was prepared as the method suggested by [7] with minor modification. Starch (13.6 gm), ethanol (85 ml), water (15 ml) and sodium hydroxide (2N) were added according to the specified pH (7.0-12.7) and reaction mixture was constantly agitated with a mechanical stirrer for 30 min at 30°C. Further, monochloroacetic acid (7.93g) was added to the mixture and incubated at temperature of 45°C with stirring for 30-120 min. The reaction was observed after specific time to obtain desired degree of substitution. The resulting product was neutralized with glacial acetic acid and carboxymethyl starch (CMS) was purified by filtration and washing with methanol-water ratio of 4:1 (v/v) until the filtrate gave negative response against silver nitrate solution. The obtained product was then dried in hot air oven at 40 °C for further experimentation.

### Determination of degree of substitution

Direct titration was used for determination of the degree of substitution of carboxymethyl starch in this study [8]. 5 g carboxymethyl starch and 150 mL acetone were mixed and 5M HCl (15 mL) was added to the dispersion and stirred for 30 min. During this process, H-CMS was formed (carboxymethyl starch in hydrogen form). H-CMS was washed several times with 80% (v/v) methanol until the solution became neutral with pH test. The neutral dispersion was filtered again, suspended in acetone, stirred for another 15 min, following which it was filtered and dried for 24 h in desiccators over silica gel. Two grams of H-CMS were dissolved in 1% (w/v) NaCl solution and was titrated with 1M NaOH. The degree of substitution was determined as follows:

$$DS = \frac{n \text{ NaOH} \times M_0}{mc - n \text{ NaOH} \times MR}$$

$$mc = mp - \left[ \frac{mp \times F}{100} \right]$$

Where,  $M_0$  = molar mass of the anhydroglucose unit = 162 g/mol; MR = molar mass of carboxymethyl residue = 58 g/mol; nNaOH = quantity of sodium hydroxide used (mol); mp = weight of polymer taken (g); mc = corrected weight of polymer (g); F = moisture (%).

### Physicochemical properties

#### Freeze-thaw stability

Native starch (2% w/v) sample was kept in boiling water for 30s with mild agitation then cooled to 25°C. CMS pastes were obtained by dispersing CMS powder in the stirring water with exhaustive mixing, the suspensions were allowed to stand overnight at room temperature (25°C) to complete swelling. 20g starch paste was added to pre-weighed 50 mL centrifuge tubes and then frozen at 18 °C in a freezer for 18 h and then thawed at room temperature (25 °C) for 6 h. Then tubes from thawing cycle of these samples were centrifuged at 4000 rpm for 30 min. The clear liquid was decanted and the residue was weighed. The percentage of syneresis (%) was then calculated as the ratio of the weight of the liquid decanted to the total weight of the paste before centrifugation.

$$\text{Syneresis (\%)} = \frac{\text{wt of liquid}}{\text{wt of initial sample}} \times 100$$

### Swelling power and solubility

Starch (0.6g) was heated with 40 ml of water to 90°C for 30 minutes. The mixture was centrifuged at 3000 rpm for 15 minute. The supernatant was carefully removed and the swollen starch sediment was weighed. Supernatant was taken in preweighed petri dish and moisture was evaporated overnight at 130°C. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. The result was expressed as:

$$\text{Solubility} = \frac{\text{wt. of soluble starch}}{\text{wt. of sample}} \times 100$$

$$\text{Swelling power(\%)} = \frac{\text{wt. of sediment paste} \times 100}{\text{wt. of sample on dry basis} \times (100 - \% \text{solubility})}$$

### Water binding capacity (WBC)

A suspension of 5g starch in 75ml distilled water was agitated for 1h and centrifuged at 3000rpm for 10min. The free water was removed from the wet starch. The wet starch was weighed and the water binding capacity is expressed as:

$$\text{Water binding capacity(\%)} = \frac{\text{wt. of residual starch}}{\text{wt. of sample}} \times 100$$

### Pasting Properties

The pasting properties of starch sample were determined using a Rapid ViscoAnalyser (RVA, Model 4D, Newport Scientific, Australia). Sample (3.0g, 14g/100g moisture basis) was weighed directly in the RVA canister and distilled water was added to obtain a sample weight of 28 g. The sample was held at 50°C for 1 min, heated to 95°C in 7.5 min, and then held at 95°C for 5 min. the rotating speed was maintained at 160 rpm along the process. The parameters obtained includes peak, final, breakdown, setback and trough viscosity were recorded.

### Morphological Properties

The structure of the starch sample was observed using a scanning electron microscope (Model S-4800, Hitachi SEM). Native starch and modified starch samples were placed in an oven at 45 °C for 5 days for dehydration before analysis. The dehydrated samples were then coated with gold powder to avoid charging under the electron beam. The granule surface and shape of starch were observed.

### Thermal Properties

By using differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) and following the standard method [9], thermal properties of starch sample were determined. The samples were prepared with a water to starch ratio of 2:1 and heated from 30 to 110°C at a rate of 10°C/min. Thermal transitions for gelatinization were characterized by T<sub>o</sub> (onset temperature), T<sub>p</sub> (peak temperature), T<sub>c</sub> (conclusion temperature) and ΔH (enthalpy of gelatinization). All enthalpy calculations were based on dry weight basis.

### Statistical analysis

All data were observed in triplicate and subjected to analysis of variance for comparative study of the experiments. Critical differences were considered to be significant at p<0.05. Statistical computations and analyses of response surface methodology were conducted using Design expert software (DX7 Trial version).

## Results and Discussion

### Effect of concentration of NaOH and reaction time on degree of substitution (DS)

The data represented in **Table 1** shows that the degree of substitution of the carboxymethyl starch increased with an increasing pH and concentration of sodium hydroxide attained a maximum degree of substitution of 0.392 at pH 9.85.

However, gelatinization occurred under this condition and the recovery of product was difficult. Thus the optimal pH observed was 7 at degree of substitution of 0.3. Response surface methodology (RSM) was used for optimize the level of pH and degree of substitution for optimized modified starch.

During the carboxymethylation process, the NaOH provides the alkaline environment for the reaction and also serving as the swelling agent to facilitate diffusion and penetration of the etherifying agent to the starch granular structure [10]. As the amount of NaOH increased, the starch gelatinized easily and the contact between starch and monochloroacetic acid was inhibited in the reaction mixture. Moreover, further increase in NaOH concentration, it diverts the monochloroacetic acid towards the side reaction. This may be occurs the final reduction in the degree of substitution at higher NaOH concentration. These results were also in accordance with findings on potato starch [11].

**Table 1** Degree of substitution of modified buckwheat starch

Sample	Time (min)	pH	DS
1	30	7	0.05
2	120	7	0.3
3	30	12.7	0.31
4	120	12.7	0.32
5	11.36	9.85	0.30
6	138.64	9.85	0.39
7	75	5.82	0.05
8	75	13.88	0.25
9	75	9.85	0.37

### **Physicochemical Properties**

#### *Freeze thaw stability*

Freeze thaw stability of the native and modified starch by evaluating the syneresis value, which was decreased on modification of starch (**Table 2**). Syneresis effect depends on degree of substitution and showing the reciprocal behavior among them. But at the maximum level of degree of substitution, the recovery of product was difficult to achieve hence so optimum value considered on the basis of more recovery is 2.99% on 0.3 degree of substitution. The improved freeze–thaw stability of carboxymethyl starch gels could be attributed to starch chain alignment retarded by incorporation of hydroxyl groups during storage which results decrease in retrogradation [12]. The syneresis values of carboxymethyl starch are in good accordance as studied by the researchers [13, 14].

**Table 2** Physicochemical properties of native and modified starch

Sample	Freeze thaw stability (%)	Swelling Power (%)	Solubility (%)	Water Binding Capacity (%)
Native Starch	22.76	9.82	9.84	110.36
Modified Starch (DS 0.3)	2.99	9.96	9.908	150.298

#### *Swelling power and solubility*

Swelling power is mainly contributed by the amylopectin content in the starch and an increase was observed with an increased in degree of modification (Table 2). This change may be due to higher hydrophilic nature of carboxyl group resulting more swelling power. Similar result was also reported in the earlier research on modified starch [11]. Solubility of the starch can be directly correlated by the amylose content present. Due to the crystalline structure of starch it is insoluble in cold water and as the pH was increased, solubility first decrease up to certain level and then increased. The increased solubility may due to increasing hydrolysis degree of the starch producing water soluble small molecules.

*Water binding capacity*

Water binding capacity of native and modified starch is represented in Table 2. Starch granules form a hydrated layer with the hydrophilic hydroxyl groups when dispersed in water. Buckwheat starch has smaller granules results increases its water binding capacity as compare to other starches [15]. Modification of starch considerably changes the water binding capacity of starch and it increases with increase in degree of saturation. The increase of pH increase the water binding capacity up to some extent and then decreases simultaneously, this effect may be due to the reduction of the amorphous region in the starch granules.

*Pasting Properties*

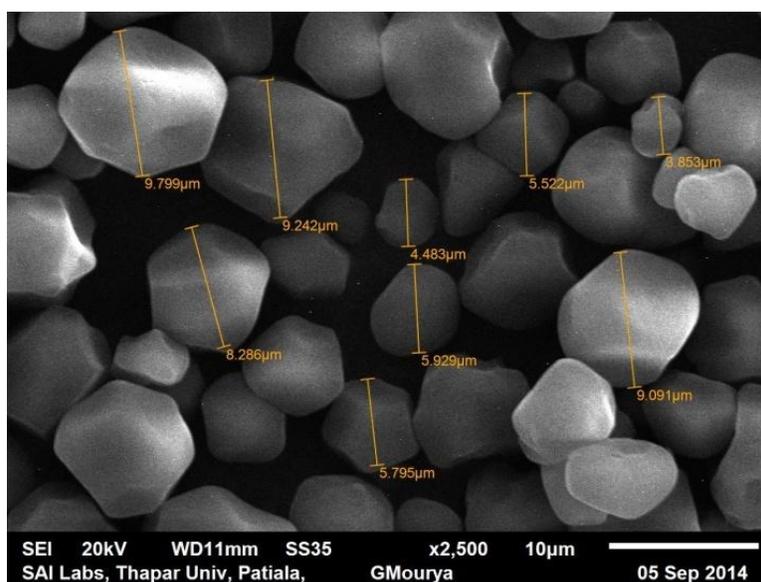
The pasting properties of the native and modified starch are shown in **Table 3**. After modification of starch, peak viscosity progressively reduced and trough viscosity is increased with increase in reaction time. This behavior of starches may be due to presence of negative charges in respective starch molecules. Breakdown and final viscosity was found increased on modification leads to increased intermolecular force in starch granules which prevent them from leaching during cooling. Due to the repulsion of negatively charged carboxymethyl groups, the starch molecules tended to exist in a more expanded state, thus occupying more space for gyration and exhibiting higher viscosity [16].

**Table 3** Pasting properties of native and modified starch

Sample	Peak Viscosity (cp)	Trough Viscosity (cp)	Breakdown Viscosity (cp)	Final Viscosity (cp)	Setback Viscosity (cp)
Native starch	6468.12	2971.51	3496.43	3494.66	2369.67
Modified Starch (DS 0.3)	4269.41	3167.28	1102.08	4974.24	1740.95

*Morphological properties*

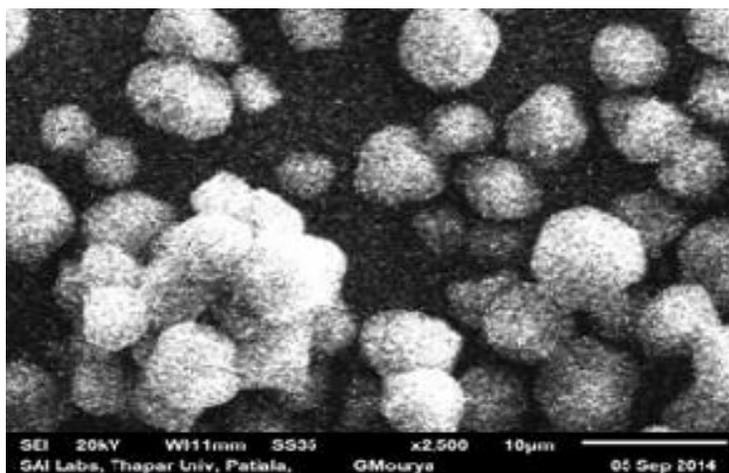
The scanning electron micrographs of native starch and modified starch are shown in **Figures 1 and 2**, respectively. The native starch granules were smooth polygonal in shape and sizes ranges from 3-9  $\mu\text{m}$ . On modification, integrity of the starch granules was still preserved but the granular surface became rough and scaly (Figure. 2). This change may be due to the loss of crystalline structure after modification of starch. Similar observations have been reported for carboxymethyl starches in previous studies [8, 10] and shown as reduction in the molecular weight may be due to molecular degradation by the alkaline treatment.



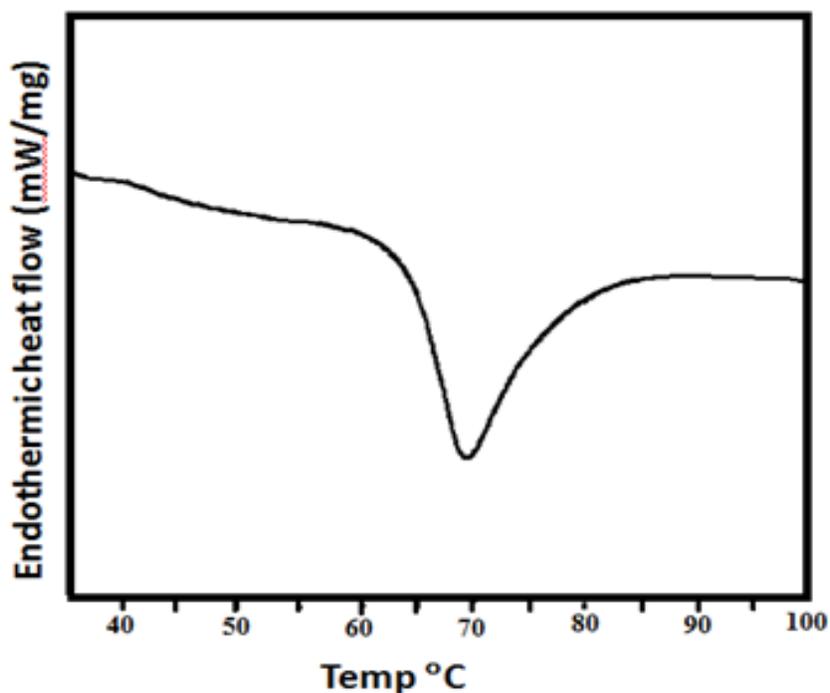
**Figure 1** Scanning electron micrographs of native buckwheat starch

*Thermal property*

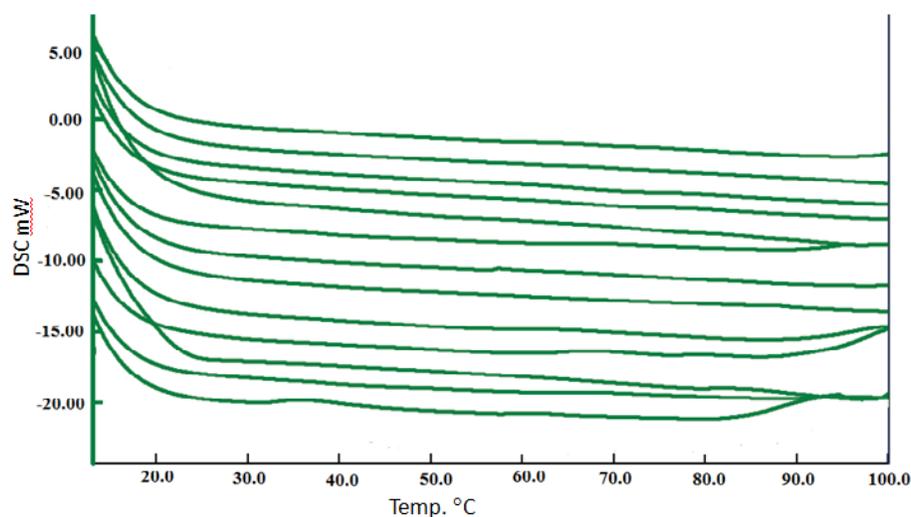
The Differential scanning calorimeter thermograms of native and modified starch was represented in **Figures 3 and 4**, respectively. Gel formation was observed in modified starch on water addition at room temperature ( $\leq 35^{\circ}\text{C}$ ). The results were confirmed by DSC thermograms as there was endothermic peak in the curves. Gelatinization enthalpy is an indicator that carboxymethylation had tremendously affected the starch crystallinity and eventually increased the starch amorphous region by destroying its crystalline structure. Similar observations have been also reported in previous studies [17, 18] for water yam and pigeon pea starch.



**Figure 2** Scanning electron micrographs of modified buckwheat starch (DS is 0.329)



**Figure 3** DSC curve for native buckwheat starch



**Figure 4** DSC curve for modified buckwheat starch

## Conclusion

Modification of starch was carried out by monochloroacetic acid at pH (7-12.7) for time (30-120) min. Response surface methodology (RSM) was used for optimize the modification parameters on the basis of degree of substitution. Modification of starch has significant effect on its physicochemical, pasting, thermal and morphological properties. Swelling power, solubility and water binding capacity parameter of starch were increased after modification. Scanning electron micrographs reflects changed behavior of the modified starch from crystalline to amorphous nature.

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