Chemical Modification and Pharmaceutical Evaluation of Starch obtained from Edible Natural Sources

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Carboxymethylation, Succinylation, Wound Healing Management, *Lens culinaris, Echonochola esculenta.*

ABSTRACT
This study investigates the carboxymethylation of starch extracted from *Echinochloa esculenta* and *Lens culinaris*, aiming to characterize their properties and evaluate their pharmaceutical potential. Fourier Transform Infrared Spectroscopy (FTIR) confirmed successful carboxymethylation, with characteristic peaks at 3399.47 cm^{-1} to 3402.81 cm^{-1} for *Echinochloa esculenta and Lens culinaris* respectively. Similarly, succinylation chemical modifications of starches were carried out for *Echinochloa esculenta and Lens culinaris*. Wound healing dressings were prepared from these succinylated starches, impregnated with giloy and turmeric extract. *In vivo* evaluation using albino rats showed a 30% faster wound closure compared to controls, demonstrating significant potential in therapeutic applications. This application on animal is the result of wound healing management by modified starch.

1. INTRODUCTION

Properties like inherent biodegradability, excessive abundance, and annual renewability, starch is one of the most potential natural polymers. Because of one's lower goods cost and their capacity to be run using conventional plastic processing equipment, starches provide an extremely appealing low-cost foundation for new biodegradable polymers[1,2,3,4]

Foods with a higher amylopectin content exhibit exceptional gelatinization and swelling properties[5]. Food with a higher amylose content exhibits the characteristics of amorphous materials. Fig. 1 and 2 shows chemical structure of starch and amylose with amylopectin. Additionally, foods with a higher concentration of amylopectin or a lesser concentration of amylose exhibit a faster rate of digestion[6].

Starch possesses distinctive functional and physicochemical properties as a result of the presence of amylose and amylopectin, and these properties vary among various food sources. It is extensively employed in a variety of industries, including paper, mining, construction, pharmaceuticals, and food, as a result of its distinctive attributes.[7]

Unmodified starches are generally less objectionable for a variety of industrial applications due to their propensity to degrade under manufacturing conditions, including high pressure and temperature, wide difference of pH, excess wear and shear rate, and mimicking freeze-thaw variation.[8]

The modification of starch is a critical component in its ubiquitous use. Chemical, biochemical, and physical modifications of starch have been implemented in the food industry. The digestibility of starch can be manipulated by the formation of a steric barrier at the site of enzymatic action as a result of chemical modification. For instance, the addition of a hydrophobic group in the form of octenyl succinate (OS) to the starch molecule to prevent starch from being cleaved by amylolytic enzymes. The method of esterification is one of the reactions of chemical modifications of starch.[9,10]

Starch, biodegradable[11] gelatinized have been implemented on excisional damage or wound on outer surface of skin for a variety of biomedical applications to restore the structure of the injured tissue. [12]
Starch, gelatinization is created by utilising hydrophilic/hydrophobic polymers that exhibit a strong affinity for water molecule. The hydrophilic nature of the polymer and the porous, three-dimensional structure of the gelatinized result in a high rate of water absorption at the application site.[13]. carboxymethylated starch was gelatinized and applied for the wound management at the place where wound was created on "rat" to observe the effect of modified starch.
The work presented in this paper is dedicated to the examination of effect of carboxymethylated starch gelatinized which was isolated from natural sources of from lentil and barnyard millet. This investigation lends credence to the potential of this starch for industrial applications.

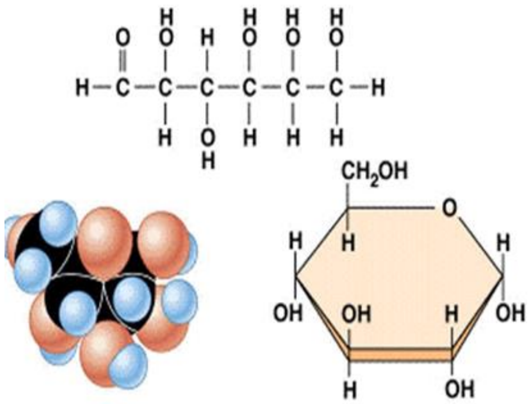

Figure 1. Chemical structure of Starch

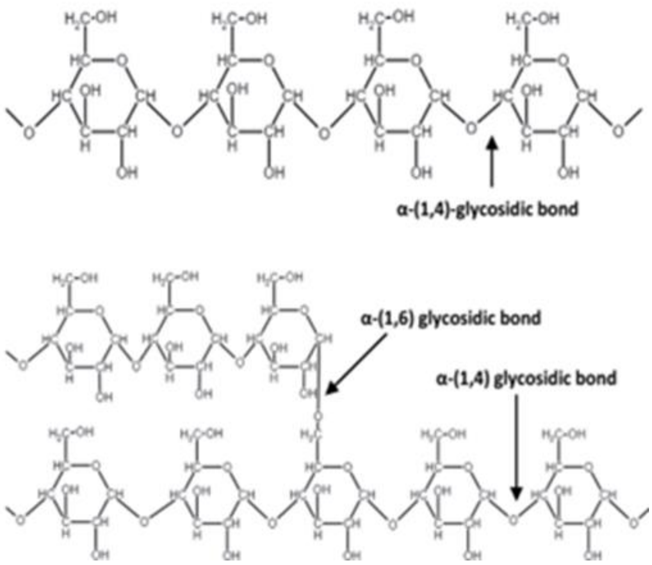

Figure 2. Chemical structure of amylose and amylopectin

Carboxymethylation is the process of introducing carboxymethyl groups[14] to starch molecules, which results in the incorporation of negative charges. This process improves the solubility and water absorption characteristics of the starch. This modification is useful in industries such as textiles, pharmaceuticals, and paper, where high solubility and film-forming capabilities are essential. The selection of carboxymethylation is based on their capacity to significantly change the functional properties of starches, thereby broadening their application spectrum [15].

2. MATERIALS AND METHODS

2.1 Materials

The seeds of Barnyard Millet (commonly known as sanwa rice) ***(Echinochloa esculenta)*** and masoor dal ***(lens culinaris)*** were purchased from Patanjali store in buddhi Vihar, Moradabad. Seed authentication was performed by CDRI-National Institute of Science Communication and Policy Research. Under this Authentication No.-NIScPR/RHMD/Consult/2023/4679-80. The authentication has been done on the basis of macroscopic studies of sample followed by matching the sample with authentic sample deposited in the RHMD.
Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Ethanol, Monochloroacetic acid were used. All the chemicals were at having a laboratory grade. Water was used in all experiments was de-ionized.

2.2 Extraction of starch

With some modifications, starch was extracted using the alkaline extraction method described by[16].In this process, *Lens culinaris and Echinochloa esculenta* were soaked (25gm) in 100 milli litre of 0.2% solution of NaOH, it was agitated for 30 minutes and stored at 4°C for 24 hours. The yellowish uppermost layer was eliminated. Following the grinding of lentil and *Echinochloa esculenta* in to a fine paste separately. Then suspend the *Lens culinaris and Echinochloa esculenta* paste in a hydrochloric acid (HCl) solution. This suspension incubated at a moderate temperature (40°C) for several hours. The resulting suspension was centrifuged to separate the starch from the other components. After rinsing the sediment with a water to remove any residual hydrochloric acid or other impurities, dry in a convection oven at 35–40 °C, the resulting slurry of starch was passed through a sieve with care using a mortar and pestle before being stored in an air tight container for future experiments.

2.3 Preparation of carboxymethyl starch

The synthesis of carboxymethylated starch was performed based on the procedure [17] of with some modifications. At room temperature, in 20 mL of solvents, 5 g of starch was added, while 5 mL of concentrated aqueous sodium hydroxide solution was added and agitated vigorously for 15 minutes. The stirring process was maintained at 40°C for an additional hour, and an equimolar quantity of monochloroacetic acid (dissolved in 6 mL of solvent which was organic) was subsequently introduced over a 15-minute period. The mixture was kept to react at 40°C for 5 hours. After the chemical modification process, the solution is kept in methanol and then subjected to acetic acid. After filtration the product was collected, rinsed many times with 75% (w/w) aq. methanol and pure methanol, and then dried at 111°C by using vacuum furnace. After that degree of substitution (DS) was calculated

2.4 Determination of degree of substitution

The determination is done using the most frequently method in the literature was direct titration, which provided a trustworthy estimate of the degree of substitution of Chemically Modified Starch[21]. Therefore, direct titration has been used to determine the degree of substitution of Chemically Modified Starch in this research. CMS weighing approximately five grams was mixed in acetone measuring 150 mL and 5 M Hydrochloric acid measuring 15 mL was added. The mixture was agitated for 30 minutes. The Chemically Modified Starch sodium form was turned into hydrogen form of carboxymethyl starch H-CMS. hydrogen form of carboxymethyl starch was rinsed with 75% (v/v) methanol on multiple times until the pH test showed that the incumbent solution was neutral. The solution was then was filtered once again,

mixed in acetone, and it was stirred for 15 minutes. Then solution was then filtered and desiccated by using silica gel for a day. A one percent (w/v) Sodium chloride was put to use to dissolve two grammes of H-CMS, which was then titrated with 1 Molar sodium hydroxide.
The degree of substitution was determined as follows:

$$DS=\frac{nNaOH \times Mo}{mc-nNaOH\times MR} \qquad m_c = m_p - [m_p x\ F/100]$$

Where Mo is called as molar mass of the anhydrous glucose whose per unit is 162g/mol; MR is called as molar mass of carboxymethyl residue = 58 gm/mol; nNaOH = quantity of sodium hydroxide used (mol); mp = weight of polymer in grams; mc = corrected weight of polymer (g); F = moisture (%)

2.5 Characterization by FT-IR

The detailed study of the surface functional groups of the starch was conducted utilising a Shimadzu FTIR-8400S. A resolution of 4 cm^{-1} for wavenumbers and an accumulation scan of 128 were implemented. An analysis of the surface functional groups was conducted within the wave number ranging from 4000 to 400 cm^{-1}

2.6 Wound Healing Activity

Similarly, CMS was carried out for succinylated starch for both *Lens culinaris* and *Echinochloa esculenta* then the wound healing activity was performed using a wound excision model [22] [23]with some modifications. Twenty-four Albino adults male Wistar rats (weighing 150–250 g) were randomly assigned to six groups, with six rodents in each group. After the rodents were anaesthetized and subsequently removal of hair from the dorsal area of each rat was done. Wound healing dressings were prepared from these succinylated starches, impregnated with giloy and turmeric extract. Then wound was inflicted using a sterile scalpel and scissors, with a diameter of approximately 1 cm. Group 1 received marketed formulation- T Bact-Mupirocin ointment (positive control), group 2 received no treatment (negative control), and group 3 received drug formulated from millet extract, group 4 received drug formulated from Lens culinaris extract. The gel was applied two times a day. The wound healing effect was assessed following the treatment on each subsequent day, and the gel was reapplied until the wound had completely healed.
The monitoring of the activity was done and two parameters were taken into consideration. One was visible and other was histopathological. Visible parameters included the rate and extent of healing until regrowth of hair. Histopathological effects were to be studied after the sacrifice of the rat. The effect of hydro-gel is shown in fig. 5 and fig. 6 at an interval of seven days for both *Lens* culinaris and *Echinochloa esculenta* starches.[24]

3. RESULTS AND DISCUSSION

The yield of starch from *Lens culinaris* was approximately **75.6%** by weight, while *Echinochloa esculenta* yielded about **80.3%.** These yields are consistent with typical starch extraction efficiencies reported for other legumes and grains. The higher yield from *Echinochloa esculenta* suggested a more efficient extraction process or a naturally higher starch content.
Carboxymethylation was verified by FT-IR, revealing new absorption bands 1600cm-1, indicative of COO- stretching vibrations. The degree of substitution value obtained was ranged from 0.20 to 0.52 and 0.18 to 0.55 for Lens culinaris and Barnyard millet respectively. Degree of substitution for CMS by carboxymethylation of Chinese yam rice starch should have values ($0.04<DS<0.46$) and for corn starch should have values ($0.4<DS<0.6$)[20].
Carboxymethylation significantly increased the solubility and water absorption capacity of starches. This modification led to the formation of clean, viscous solutions, which are highly desirable in pharmaceutical and paper industries where high solubility is required.

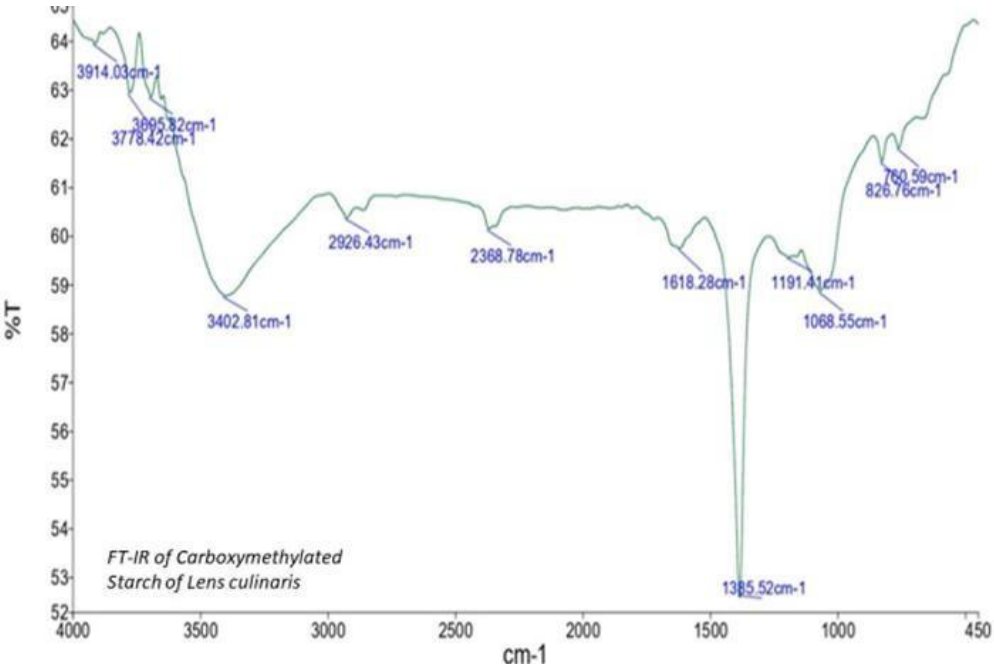

Figure 3. FTIR of Carboxymethylated starch of *Lens culinaris*

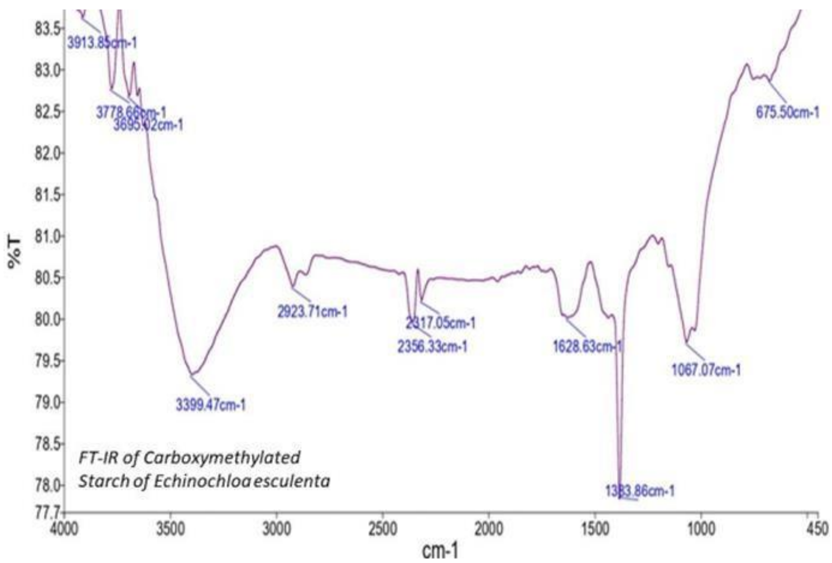

Figure 4. FTIR of Carboxymethylated starch of *Echinochloa esculenta*

In Carboxymethylated *Echinochloa esculenta* Starch, this band shifted slightly to 3399.47 cm^{-1} and in *Lens culinaris* 3402.81 cm^{-1} due to the carboxymethylation process, which introduces additional –OH and –COO^{-} groups. Both native and carboxymethylated EEs starch have C-H stretching vibrations around 2800-3000 cm^{-1}. However, these bands are not significantly altered by carboxymethylation. Native rice starch lacks a strong carbonyl (C=O) stretching absorption band because it does not have significant carbonyl functionality. But, in Carboxymethylated Starch, A new absorption band around 1628.63 cm^{-1} appear, corresponding to the stretching vibration of the carboxylate (COO^{-}) group introduced by carboxymethylation. Native rice Starch Shows characteristic bands for carbon-oxygen-carbon and CO stretching in the range of 1000-1150 cm^{-1} due to the glycosidic linkages and C-OH groups in the glucose units, very particularly at 1154.76 cm-^{1} : C-O stretching vibration of glycosidic bonds, 1080.52 cm-^{1} : C-O stretching vibration of a primary alcohol group and 1019.26 cm-^{1} : C-O stretching vibration of a secondary -OH group. But, in carboxymethylated starch, the peak for C-O stretching vibration of a 1^0 -OH group is shifted due to the introduction of carboxymethyl groups to 1067.07 cm^{-1}

Animal study

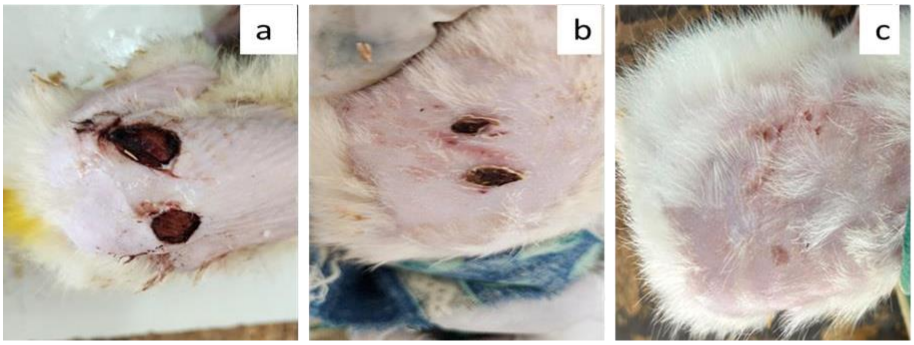

Figure 5. Wound healing models (a) Day-0 (b) Day-7 (c) Day-14 of modified starch *Lens culinaris*

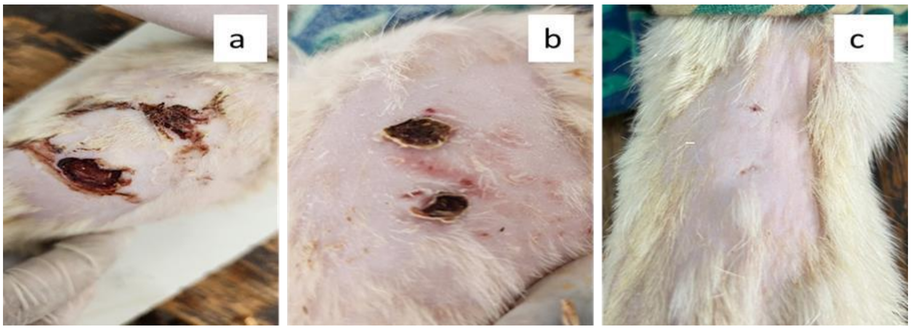

Figure 6. Wound healing models (a) Day-0 (b) Day-7 (c) Day-14 of modified starch *Echinochloa esculenta*

It can be seen that excision wound diameter is decreasing as time passes by. With passage of each week, the diameter gets smaller. Both the Succinylated CMS obtained from *Lens culinaris* and *Echinochloa esculenta* act as good gelatinising agent for greater delivery of constituents in formulation. The study is not comparative in nature but demonstrates that CMS derived from both natural sources are good source of polymers which show almost no visible adverse effects in wound healing activity. It showed more promising effects than marketed formulation.

Histopathological study

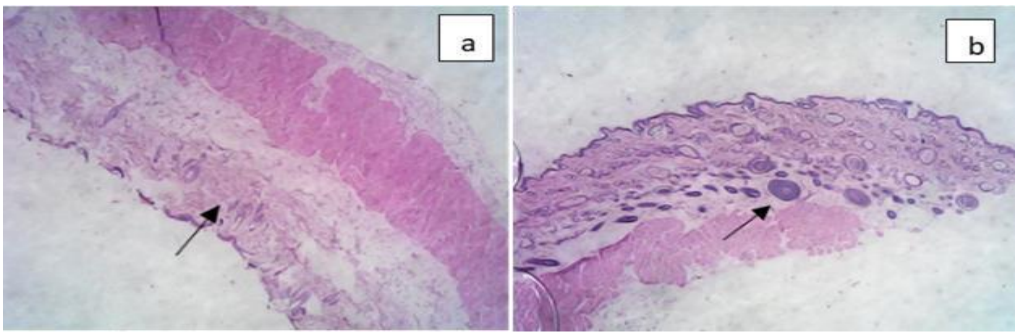

Figure 7. Histopathological slide of both sources (a) *Lens culinaris*

(b) *Echinochloa esculenta*

The histopathology slide demonstrates the presence of granulation tissue, collagen deposition, and re-epithelialization, all of which are indicative of natural wound healing in albino rats. Furthermore, the wound is in the proliferative to remodelling phase of healing, as evidenced by the organised tissue structure and moderate inflammatory response

4. CONCLUSION

The starch was extracted and then subjected to chemical modification, which was indeed successful as demonstrated by Infrared spectroscopy. The chemically modified starch (CMS) was then put to animal study on Wistar albino rats. The study was coherent of the fact that wound healing activity of the CMS has some promising scope. The CMS can act as a good substitute for synthetic polymer. The CMS showed properties which is not found in synthetic polymer, which fewer side-effects, as it is derived from natural source. It is also consistent with the psychological behaviour of Indian patients that it is not obtained from animal origin and thus can be put to commercial aspects. The histopathological studies are in line with a developing formulation which elaborated that marketed formulation is not as effective as developed CMS.

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