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Research Article

PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYL MILLET STARCH AND PREGELATINIZED MILLET STARCH

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ABSTRACT

Objectives: The aim of this study was to prepare and characterize the physical and chemical characteristics of carboxymethylated millet starch (CMMS) and pregelatinized millet starch (PGMS) to evaluate their improved applications over native millet starch.

Methods: CMMS and PGMS were prepared and assessed for various physicochemical properties. Tests were conducted for moisture content, moisture uptake, pH, amylose content, swelling capacity, hydration capacity, degree of substitution (DS), and micromeritic properties using standardized techniques.

Results: The measured properties for CMMS and PGMS were as follows: Moisture content was 4.2% and 4.8%; moisture uptake was 32.4% and 12.3%; pH was 7.6 and 6.3; and amylose content was 14.7% and 19.9%, respectively. CMMS had a DS of 0.20 and was completely soluble in water, while PGMS exhibited a swelling capacity of 4.7, a hydration capacity of 6.8, and demonstrated good flow and compressibility.

Conclusion: Both CMMS and PGMS exhibit enhanced properties that make them suitable for broader applications in pharmaceutical formulations than native millet starch.

Keywords: Carboxymethyl millet starch, Modification, Physicochemical characteristics, Pregelatinized millet starch.

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INTRODUCTION

Native starch has limited applications due to its high molecular weight, poor solubility in water, the instability of the viscous solutions it forms, and its vulnerability to microbial contamination [1]. These innate properties could be modified using various physical, chemical, and genetic techniques to produce starch with a variety of functional characteristics that would aid in a variety of specialized applications [2-5]. Physical (heat-treatment) techniques are carried out for starch modification because they are significantly less expensive and do not involve chemical reagents [2]. One of these techniques is pregelatinization, which is precooking to produce starch that is easily dispersed in cold water and forms relatively stable suspensions [6]. Pregelatinized starch shows higher swelling power, solubility, and water absorption capacity when compared with its native counterpart, this is related to the breaking of cohesive bonds (inside the starch) and leaching of the amylose within the process of gelatinization [7]. The previous study showed that pregelatinized starch can undergo plastic distortion because of the compaction process [8]. To create carboxymethyl starch (CMS), the hydrogen in the starch can be replaced with another substance, such as a carboxymethyl group. Adding such a large functional group reduced the starch's tendency to recrystallize and made it more resistant to heat and bacterial degradation. Low gelatinization temperature, high swelling capacity, and cold water solubility are among CMS's major properties [9]. Grains such as rice, wheat, millet, and maize are some sources of starch as well as some root vegetables such as potatoes [7]. This polymeric carbohydrate can amount to up to 70% of the millet seed, making it one of the primary sources of starch. Interestingly, millet became a staple food for humans 10,000 years ago before the rise of rice and wheat [10]. Although this crop has gained popularity because of its nutrient profile and health benefits, it is still regarded as being understudied [11]. *Pennisetum typhoides* (Burm.f.) Stapf.f.ex Hubbard often known as pearl millet belongs to the *Poaceae* family and is a major substance crop in Sudan with a sizable annual production. Millet production in Sudan was predicted at 954,000 tons in 2017/2018. With such a large supply, the local need for starch in the pharmaceutical industry would be largely satisfied by a millet-derived pharmaceutical excipient with requisite properties. Our study aimed to prepare and characterize the physicochemical properties of carboxymethylated millet starch (CMMS) and pregelatinized millet starch (PGMS).

MATERIALS AND METHODS

Materials

A sample of millet seeds was purchased from Abnaa Sayed Elobied Agro Export Company, Khartoum, Sudan, and authenticated at the National Research Center in Khartoum City, Sudan. Materials such as standard and amylose were purchased from Aladdin in China. Other materials such as xylene, sodium hydroxide, iodine, potassium hydroxide, and acetone were obtained from SD fine-Chem Limited, India. Potassium iodide was obtained from Scharlau, Spain. Ethanol, methanol, and hydrochloric acid were products of BDH Limited Poole, England. Chloroacetic acid and citric acid were obtained from Matlab, UK, and Oxford laboratory, India, respectively.

Synthesis of PGMS

Pregelatinization of starch was done according to the method described in the literature [1], with slight modifications. In a digital water bath, 50 g of starch in 250 mL of distilled water was heated to 90°C for 15 min with constant stirring. The resulting pastes were spread out onto trays made of stainless steel and allowed to dry at room temperature for a full day. The flakes were ground in a blender, sieved through a 180-µm sieve, and kept until needed.

Synthesis of CMMS

The CMMS was synthesized according to the method described by Varghese and Komala, 2022 [9], with slight modifications. First, 60 g of monochloroacetic acid was dissolved in 800 mL of water. With continuous stirring, 200 g of millet starch was dispersed in the solution. After adding 200 mL of a 30% w/v aqueous NaOH solution to the mixture, it was heated for 1 h to 70°C. Then, the obtained slurry was treated with 50% citric acid, filtered, and washed with 95% ethanol to achieve purification. The obtained modified CMMS was air-dried.

Degree of substitution (DS) of CMMS

The technique created by Li *et al*. 2010 [12] was used to measure the DS of CMS. After dispersing 5 g of CMMS in 150 mL of acetone, 5 M of HCl was added, and the mixture was agitated for 30 min. During that process, the CMMS, which was sodium-based, changed into H-CMMS, or CMMS in hydrogen form. After that, the H-CMMS solution was rinsed 4 times using 80% (v/v) methanol until neutral. After filtering, the dispersion was suspended in acetone, filtered again, and then, dried by a desiccator over silica gel for 24 h. Two grams of H-CMMS were dissolved in a 1% (w/v) NaCl solution, and titration was performed using a 1M NaOH solution. Measurement of DS was done according to the equation below:

$$DS = \frac{Q_{NaOH} X M_g}{W_{ds} - Q_{NaOH} X M_R}$$

Where

Q NaOH: Quantity of sodium hydroxide used per moL.

Mg: Molar mass of the anhydrous glucose units = 162 g/moL.

Wds: Weight of starch taken on a dry basis (g).

MR: Molar mass of carboxymethyl residue = 58 g/moL.

Moisture content

Using a moisture analyzer, the moisture content of the starch was determined.

pH

This was determined using a calibrated pH meter and the methodology described in reference [13].

Swelling capacity of starch

The method of Nataraj and Reddy, 2020 [14] was used to calculate the swelling power. Every 5 g of starch sample (V1) had its tapped volume measured and recorded. Next, the starch powder was mixed in distilled water (100 mL). The volume occupied by the sediment (V2) was recorded after 24 h and the swelling capacity was calculated as follows.

$$\text{Swelling capacity} = \frac{V2}{V1}$$

Hydration capacity

In a centrifuge tube, 2 g of each starch powder (Y) was placed and 10 mL of distilled water was added. The tube was shaken intermittently for 2 h and centrifuged for 10 min. Following centrifugation, the weight (X) of the powder was calculated after the supernatant was decanted. The measurements were carried out in triplicate and the hydration capacity was calculated according to the following equation [14].

$$\text{Hydration capacity} = \frac{X}{Y}$$

Measurement of moisture sorption capacity

The moisture sorption capacity was determined using the method described in references [14,15] with minor modifications. Five grams of each type of starch were equally distributed among Petri dishes and kept at room temperature in a desiccator with a relative humidity of 75% using a saturated NaCl solution. After 48 h, the samples were removed and an increase in weight was noted. The percentage weight gain was used to calculate the moisture sorption capacity.

Determination of amylose/amylopectin ratio

The amylose-amylopectin ratio of starch was measured by a calorimetric method established by Abdalla *et al*., 2009 [16]. In a 25 mL volumetric flask, 5 g potassium iodide and 0.5 g iodine were placed and diluted with water (stock A). A volumetric flask containing 2.5 mL of stock A was diluted to a 25 mL with distilled water (reagent B). In a 100 mL beaker, starch samples (0.02 g) were placed, 10 mL of 0.5N KOH solutions were added, and the starch was dispersed under magnetic stirring for 3 min. The dispersed samples were put in a 100 mL volumetric flask and distilled water was added. Then, 10 mL of starch dispersion was taken into a 50 mL volumetric flask, followed by 5 mL 0.1N HCL and 0.5 mL of iodine (reagent B). Finally, the volume was diluted to 50 mL to measure the blue color's absorbance at 625 nm using an ultraviolet -visible spectrophotometer. Calibrations of the amylose colorimetric test increments from 2 to 12 mg were plotted against absorbance at a wavelength of 625 nm.

Micromeritic properties

The micromeritic properties of starch samples were evaluated using the method described [17]. In addition, the following equations were used to calculate the Hausner's ratio and the Carr's index from the bulk and tapped densities:

$$\text{Carr index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Scanning electron microscope

The surface morphology of CMMS and PGMS granules was examined using a scanning electron microscope in accordance with the protocol mentioned [18]. The test specimens were attached to stubs with double-sided tape. The sample was fixed, covered with a thin layer of gold, and investigated under a vacuum. To ensure clear images, scanning electron microscopic images were taken at various magnifications.

Statistical analysis

Data were expressed as mean±standard deviation and were analyzed by one-way analysis of variance followed by Tukey honestly significant difference test for multiple comparisons.

RESULTS

Physicochemical and micromeritic characteristics of CMMS and PGMS

Table 1 shows the physiochemical and micromeritic characteristics of CMMS and PGMS. CMMS and PGMS demonstrated improved physicochemical properties. CMMS showed high solubility in water, a pH of 7.6, and a moisture uptake of 32.4%. PGMS had a pH of 6.3, higher swelling (4.7) and hydration capacity (6.8), and better moisture stability (uptake: 12.3%). Both starches exhibited favorable flow and compressibility, indicating potential for pharmaceutical applications.

Scanning electron microscope

Figs. 1 and 2 present the scanning electron microscopy (SEM) images for CMMS and PGMS, respectively. SEM images revealed that the granules of both modified starches were large, amorphous, and smooth, further supporting their suitability for compaction in tablet formulations.

DISCUSSION

In pharmaceutical manufacturers, CMS is commonly employed as a tablet disintegrant; however, low substituted (DS up to 0.3) polymer, which is partially cross linked, is primarily used for that purpose and is referred to as sodium starch glycolate [19]. The DS of CMMS was found to be 0.20, which increases hydrophilicity and enhances the ingredient's ability to hold water, making it more soluble in aqueous systems because it adds carboxymethyl groups to molecules' surfaces [20]. As shown

Table 1: Physicochemical and micromeritic characteristics of CMMS and PGMS

Parameters	CMMS*	PGMS*
Moisture content (%)	4.2±0.25	4.8±0.01
pH	7.6±0.15	6.3±0.75
Swelling capacity	**	4.7±0.27
Hydration capacity	**	6.8±0.56
Moisture uptake (%)	32.4±1.25	12.3±0.49
Amylose (%)	14.66±0.28	19.88±0.92
Bulk density	0.81±0.02	0.67±0.05
Tapped density	0.94±0.02	0.74±0.08
Carr's index (%)	10±1.2	9.46±1.38
Hausner's ratio	1.1±0.05	1.1±0.08
True density	1.54±0.05	1.46±0.02
Angle of repose(°)	22.9±0.52	29.1±1.9

CMMS: Carboxymethylated millet starch, PGMS: Pregelatinized millet starch. *All values are expressed as mean±standard deviation, n=3. **CMMS: Is completely dissolved during swelling capacity and hydration capacity tests

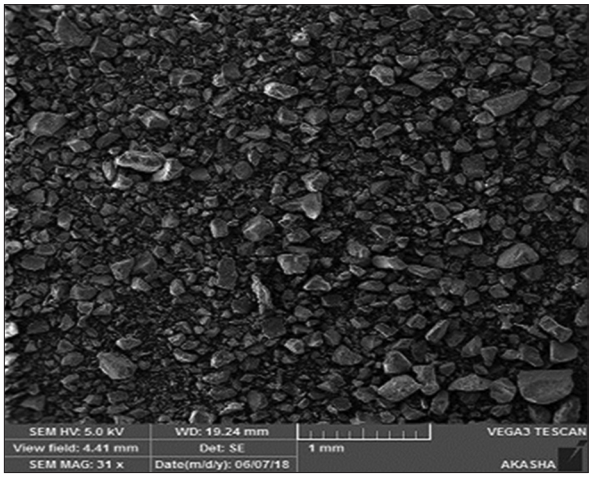

Fig. 1: Scanning electron microscopy image of carboxymethylated millet starch at ×31 magnification, showing large and amorphous granules (210–240 µm) with smooth surfaces and no observable cracks

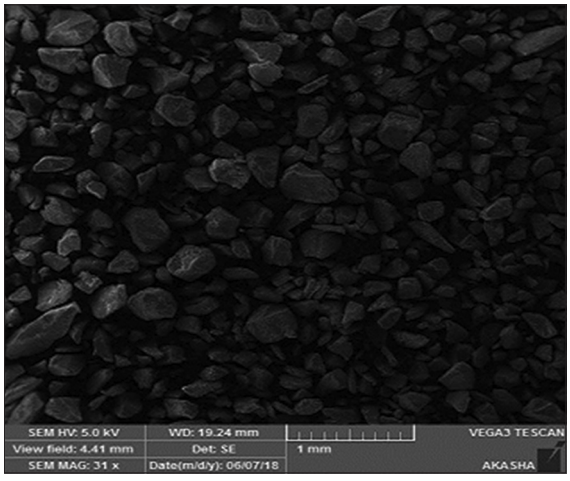

Fig. 2: Scanning electron microscopy image of pregelatinized millet starch at ×31 magnification, showing large and amorphous granules (230–260 µm) with smooth surfaces and no observable cracks

in Table 1, the pH and water content of two types of modified starch were within the acceptable limits of the British Pharmacopeia [21]. The swelling ability of starch granules is an important characteristic because the swelling power is not only a measure of the hydration capacity of the sample but is also an indication of the associative forces in the granules [22]. While CMMS was soluble and formed transparent viscous solution in cold water, PGMS was found to have higher swelling and hydration capacity than the native one, which indicated better disintegrant abilities of the former compared to the native one. The relative physical stability of tablets made from a substance under humid storage conditions indicates their moisture sorption capacity, which is used as a measure of the substance's moisture sensitivity [14]. The higher the rate of moisture sorption the higher will be the deteriorating effect on drugs that undergo hydrolytic decomposition, in terms of moisture uptake. CMMS demonstrated higher moisture uptake when compared with PGMS, suggesting that CMMS should be avoided with moisture-sensitive substances. Researchers emphasized the function of amylose in swelling resistance and solubility of granules; because swelling progressed rapidly after amylose molecules were leached, the amylose percentage of the modified starch was less than natural starch, indicating that the modified starch had better swelling and solubility properties. Since amylose provides gel strength and amylopectin contributes to high viscosity, the quantity of amylose in the granules significantly influences the functional and physicochemical characteristics of starch and can differ within the same botanical variety [23,24]. The main factors affecting bulk density are particle size, particle size distribution, and particle shape. This parameter characterizes the packing behavior of powder during the different tableting unit operations, including die filling, mixing, granulation, and compression. It is an indirect measure of powder flow. The ability of a material to decrease volume under pressure is measured by Carr's index and Hausner's ratio, which also indicates the expected flow behavior of granules when compressed to create a compact mass [25]. Tablets with greater weight variation result from a decrease in powder flow as these indices' values rise [26]. The tendency of granulated or powdered materials to flow, for instance, from hoppers through the feed frame into tableting machines, could be determined using the angle of repose [27]. Micromeritic results illustrated that the modified starch has good flow and compressibility properties. Particle compaction and flow characteristics are greatly influenced by their shapes. Particle irregularity promotes higher bond formation and increased interparticular contact [28]. CMMS and PGMS as shown in Figs. 1 and 2 possessed large and amorphous granules that ranged in size from 200 to 260 µm and had smooth, crack-free surfaces, indicating the compounds' good flow ability and compaction properties.

CONCLUSION

Multiple physicochemical properties, including water solubility, water sorption capacity, and flow ability, can be improved by carboxymethylation and pregelatinization modifications of millet starch. This would open up new avenues in a variety of fields and result in more varied and extensive uses for the modified starch. This study demonstrated that two modified millet starch species, CMMS and PGMS, could serve as viable substitutes for several widely used excipients. This will benefit the crop's additional value and aid in the pharmaceutical industry's localization in Sudan.

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AUTHORS' CONTRIBUTION

Yusra Ahmed contributed to the conceptualization, methodology, data curation, and drafting of the original manuscript. Abdullah H. Maad contributed to the formal analysis and critical revision of the manuscript. Layth Abdullah Zainy and Hassan Ali Hassan participated in the investigation, resource provision, and data collection. Zuheir Osman was responsible for supervision, validation, visualization, and project administration.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

1. Sathya E, Sekar T. Nutritional and gas chromatography-mass spectrometry analysis of *Andrographis lawsonii* gamble. Asian J Pharm Clin Res. 2021;15(1):47-51. doi: 10.22159/ajpcr.2022.v15i1.43665
2. Nawaz H, Waheed R, Nawaz M, Shahwar D. Physical and chemical modifications in starch structure and reactivity. In: Emeje M, editor. Chemical Properties of Starch. London: IntechOpen; 2020. doi: 10.5772/intechopen.88870
3. Lemos PV, Marcelino HR, Cardoso LG, Souza CO, Druzian JI. Starch chemical modifications applied to drug delivery systems: From fundamentals to FDA-approved raw materials. Int J Biol Macromol. 2021;184:218-34. doi: 10.1016/j.ijbiomac.2021.06.077, PMID 34144062
4. Niu L, Liu L, Zhang J, Scali M, Wang W, Hu X, *et al*. Genetic engineering of starch biosynthesis in maize seeds for efficient enzymatic digestion of starch during bioethanol production. Int J Mol Sci. 2023;24(4):3927. doi: 10.3390/ijms24043927, PMID 36835340
5. Odeku OA, Picker-Freyer KM. Evaluation of the material and tablet formation properties of modified forms of *Dioscorea* starches. Drug Dev Ind Pharm. 2009;35(11):1389-406. doi: 10.3109/03639040902960185, PMID 19832640
6. Nakorn KN, Tongdang T, Sirivongpaisal P. Crystallinity and rheological properties of pregelatinized rice starches differing in amylose content. Starch. 2009;61(2):101-8. doi: 10.1002/star.200800008
7. Alcázar-Alay SC, Meireles MA. Physicochemical properties, modifications and applications of starches from different botanical sources. Food Sci Technol (Campinas). 2015;35(2):215-36. doi: 10.1590/1678-457X.6749
8. Maarschalk KV, Vromans H, Groenendijk W, Bolhuis GK, Lerk CF. Effect of water on deformation and bonding of pregelatinized starch compacts. Eur J Pharm Biopharm. 1997;44(3):253-60. doi: 10.1016/S0939-6411(97)00128-8
9. Varghese N, Komala M. Analysis of *in vitro* disintegration and dissolution effect of *Cucurbita maxima* Starch In losartan FDT. Int J App Pharm. 2022;14(4):163-70. doi: 10.22159/ijap.2022v14i4.43852
10. Martin L, Messager E, Bedianashvili G, Rusishvili N, Lebedeva E, Longford C, *et al*. The place of millet in food globalization during late prehistory as evidenced by new bioarchaeological data from the Caucasus. Sci Rep. 2021;11(1):13124. doi: 10.1038/s41598-021-92392-9, PMID 34162920
11. Zhu F. Structure, physicochemical properties, and uses of millet starch. Food Res Int. 2014;64:200-11. doi: 10.1016/j.foodres.2014.06.026, PMID 30011641
12. Li X, Gao W, Huang L, Wang Y, Huang L, Liu C. Preparation and physicochemical properties of carboxymethyl *Fritillaria ussuriensis* Maxim. starches. Carbohydr Polym. 2010;80(3):768-73. doi: 10.1016/j.carbpol.2009.12.025
13. Bordoloi BJ, Kalita B, Shil D. Properties, pharmaceutical application and various technique of chemical modification in native starch: A descriptive review. Int J Curr Pharm Res. 2019;11(4):54-9. doi: 10.22159/ijcpr.2019v11i4.34950
14. Nataraj D, Reddy N. Chemical modifications of alginate and its derivatives. Int J Chem Res. 2020;4(1):1-17. doi: 10.22159/ijcr.2020v4i1.98
15. Muazu J, Musa H, Isah A, Bhatia P, Tom G. Extraction and characterization of Kaffir potato starch: A potential source of pharmaceutical raw material. J Nat Prod Plant Resour. 2011;1(2):41-9.
16. Abdalla AA, Ahmed UM, Ahmed AR, El Tinay AH, Ibrahim KA. Physicochemical characterization of traditionally extracted pearl millet starch (Jir). J Appl Sci Res. 2009;5:2016-27.
17. Ahmed Y, Maad AH, Hassan HA, Abdallah DB, Yousef M, Kadhum AM, *et al*. Evaluation of carboxymethyl millet starch and pregelatinized millet starch as pharmaceutical excipients using factorial experimental designs. Int J App Pharm. 2025;17(2):321-8. doi: 10.22159/ijap.2025v17i2.52161
18. Polesi LF, Sarmento S, Dos Anjos C. Composition and characterization of pea and chickpea starches. Braz J Food Technol. 2011;14(1):74-81. doi: 10.4260/BJFT2011140100010
19. Spychaj T, Wilpiszewska K, Zdanowicz M. Medium and high substituted carboxymethyl starch: Synthesis, characterization and application. Starch-Stärke. 2013;65(1-2):22-33. doi: 10.1002/star.201200159
20. Choi KO, Yang SC, Kim DE, Kang WS, Shin M, Choi YH, *et al*. Improving solubility through carboxymethylation of different-sized endosperm, bran, and husk rice powders. Food Sci Biotechnol. 2009;18(6):1439-46.
21. British Pharmacopoeia Commission. British Pharmacopoeia 2020. London, UK: The Stationery Office; 2019.
22. Cornejo-Ramírez YI, Martínez-Cruz O, Del Toro-Sánchez CL, Wong-Corral FJ, Borboa-Flores J, Cinco-Moroyoqui FJ. The structural characteristics of starches and their functional properties. CyTA J Food. 2018;16(1):1003-17. doi: 10.1080/19476337.2018.1518343
23. Maad AH, AL-Gamli AH, Shamarekh KS, Refat M, Shayoub ME. Antiproliferative and apoptotic effects of *Solenostemma argel* Leaf extracts on colon cancer cell line HCT-116. Biomed Pharmacol J. 2024;17(3):1987-96. doi: 10.13005/bpj/3001
24. Bashir K, Aggarwal M. Physicochemical, structural and functional properties of native and irradiated starch: A review. J Food Sci Technol. 2019;56(2):513-23. doi: 10.1007/s13197-018-3530-2, PMID 30906009
25. Okpanachi GO, Musa H, Isah AB. Evaluation of disintegrating property of native and microcrystalline starches derived from *Digitaria iburua*. West Afr J Pharm. 2013;24(1):64-71.
26. Murase Y, Takayama K, Uchimoto T, Uchiyama H, Kadota K, Tozuka Y. Prediction of tablet weight variability from bulk flow properties by sparse modeling. Powder Technol. 2022;407:117681. doi: 10.1016/j.powtec.2022.117681
27. Elballa W, Salih M. Influence of partially and fully pregelatinized starch on the physical and sustained-release properties of Hpmc-based ketoprofen oral matrices. Int J Pharm Pharm Sci. 2022;14(8):29-34. doi: 10.22159/ijpps.2022v14i8.45031
28. Khudhur ZO, Maad AH, Ghanimi H, Abdolmaleki A. Fullerene nanoparticle as new therapeutic agent for the nervous system disorders. Nanomed J. 2024;11(4):342-59. doi: 10.22038/nmj.2024.78043.1903