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Physicochemical, *in vitro* digestibility and functional properties of carboxymethyl rice starch cross-linked with epichlorohydrin

Ornanong S. Kittipongpatana, Nisit Kittipongpatana *

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

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ABSTRACT

Cross-linked carboxymethyl rice starches (CL-CMRSs) were prepared from reactions between native rice starch and varied concentrations (0.1–15% w/w, M-0.1 to M-15) of epichlorohydrin (ECH) in a simultaneous carboxymethylation-crosslinking reaction setup using methanol as the solvent. The degree of carboxymethyl substitution was between 0.24 and 0.28, while apparent amylose contents were lowered due to modification. SEM images showed minor change on the granule surface, while XRD profiles indicated slight loss of crystallinity. DSC thermograms revealed no transition peak in all treated samples. The water uptake (WU), swelling volume (SV) and free swelling capacity (FSC) of CL-CMRSs increased significantly as a result of the modification, while swelling of CMRSs cross-linked with 2% (M-2) and 3% (M-3) ECH yielded FSC values and WU values that were much greater than those of native starches and were comparable to that of Explotab®. All modified starch samples showed increased amount of rapidly digestible starch (RDS), while cross-linking with 5–7.5% ECH raised the resistant starch (RS) content, compared to native starch. M-2 also showed promising results in tablet disintegration test. ECH–CL–CMRSs could potentially be used as an excipient in pharmaceutical and food/food supplement products.

1. Introduction

The utilization of modified starches in food and pharmaceutical industries has continued to increase thanks to their broadened and improved physico-mechanical and functional properties compared to native starches. The use of cross-linking agents, such as sodium trimetaphosphate (STMP), phosphoryl chloride ($POCl_3$), adipic and acetic mixed anhydride and epichlorohydrin (ECH), has been one of the major strategies in the modification of starch. ECH, in particular, has been widely used as a cross-linking agent in not only starch but also several other biopolymers (Chang, Duan, Cai, & Zhang, 2010; Keppeler, Ellis, & Jacquier, 2009; Machín, Isasi, & Vélaz, 2012). Cross-linkage generally strengthens the hydrogen bonds in starch molecules and enhances the stability of the granules, therefore altering the physical and thermal properties of starch (Neelam, Vijay, & Lalit, 2012). Different types of cross-linking agents react variably with –OH groups on different positions of AGU. Their penetration ability into the granules is also reported to be different (Huber & BeMiller, 2001; Rodriquez-Marin, Núñez-Santiago, Wang, & Bello-Pérez, 2010), and plays an important role in the differences in both the structure and the properties/functionality of the products (Carmona-Garcia, Sanchez-Rivera, Méndez-Montealvo, Garza-Montoya, & Bello-Pérez, 2009). Cross-linking of starch with ECH, yielding distarch glycerols, has been reported in wheat (Ačkar, Babić, Šubarić, Kopjar, & Miličević, 2010), banana (Rodríquez-Marin, Núñez-Santiago, Wang, & Bello-Pérez, 2010) and corn (Ispas-Szabo Ravenelle, Hassan, Preda, & Mateescu, 2000). ECH-cross-linked high amylose corn starch was reportedly used as a controlled-release excipient for monolithic dosage forms.

The cross-linking reaction can be carried out either solely or in combination with another modification e.g., hydroxypropylation (Hung & Morita, 2005), acetylation (Raina, Singh, Bawa & Saxena, 2006) or carboxymethylation (Kittipongpatana, Chaitep, & Kittipongpatana, 2010). The dual modification is commonly employed to obtain products with optimized or desired properties. Carboxymethyl starch (CMS), an etherified starch derivatives, represents one of the most well-researched modified starches and has been approved for use in food and pharmaceutical products (Heinze & Koschella, 2005). The safety and versatile application of CMS (Spychaj, Wilpiszewska, & Zdanowicz, 2013) made this modified starch a preferred candidate for dual modification. The conditions and procedure used in CMS preparation also allow a convenient incorporation of either sequential or simultaneous cross-linking reaction, yielding a dual-modified starch. Carboxymethylated starches cross-linked with ECH have been reported for corn, potato and cassava starches (Cao, Zhou, & Bao, 2009; Onofre, Wang, & Mauromoustakos, 2009; Wang, Chen, Yaun, & Wang, 2008), but, to date, not for rice starch. A recent study on comparing the functionality of rice, corn and wheat starches as pharmaceutical

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* Corresponding author. Tel.: +66 53 944397.
E-mail address: nisitk@gmail.com (N. Kittipongpatana).

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excipients has shown that rice starch possessed the best flow, hydration capacity and swelling capacity and, even in the native form, could be used as a tablet disintegrant (Olayemi, Oyi, & Allagh, 2008). In the previous work, carboxymethyl rice starches cross-linked with STMP were prepared and their property as tablet distintegrant was reported (Kittipongpatana et al., 2010). The objective of this work was to evaluate the effects of ECH and its concentration on the physicochemical, digestibility and functional properties of dual modified cross-linked carboxymethyl rice starch (CL-CMRS).

2. Materials and methods

2.1. Materials

Native rice starch (RS) was purchased from Thai Flour Industry Co., Ltd (Bangkok, Thailand). Monochloroacetic acid (MCA) and epichlorohydrin (ECH) were products of Merck Schuchardt OHG (Hohenbrunn, Germany) and Sigma–Aldrich (Steinhiem, Germany), respectively. All other chemicals used in the preparation and analysis of modified starches were of AR grade or equivalent. Double-distilled commercial grade methanol (MeOH) was used in the washing of the modified starches, with the exception of the final wash in which the AR grade methanol was used.

2.2. Preparation of CMRS and CL-CMRSs

MCA (40 g) was dissolved in 254 g MeOH. Then, while stirring, 138 g of rice starch powder was added into the solution followed by 78.5 g of 50% w/w NaOH solution. Varied amounts of ECH (0, 0.1, 0.5, 1, 2, 3, 5, 7.5, 10, 12.5, 15% w/w of starch) were then added into the reaction. The mixture was heated to, and maintained at, 70 °C for 60 min with continuous stirring. At the end, the reaction was terminated by neutralization with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 80% methanol until the filtrate gave no precipitate when tested with silver nitrate solution and a final wash with 100% methanol. The products, designated as M-0 (CMRS), M-0.1, M-0.5, M-1, M-2, M-3, M-5, M-7.5, M-10, M-12.5 and M-15 (CL-CMRSs), were oven-dried at 50 °C for 6 h before passing through sieve No. 60 (60 mesh, 0.250 mm).

2.3. Degree of substitution (DS)

The DS of carboxymethyl group in the samples was determined by a two-step analysis, which included titration and residue on ignition. The D.S. can be calculated using the equation;

$$\text{D.S.} = \text{A} + \text{S} \tag{1}$$

when A is the degree of substitution of carboxymethyl acid and S is degree of substitution of sodium carboxymethyl. A and S can be calculated using the information from the titration and ignition steps;

$$\text{A} = 1150\text{M}/(7120 - 412\text{M} - 80\text{C}) \tag{2}$$

$$\text{S} = (162 + 58\text{A})\text{C}/(7120 - 80\text{C}) \tag{3}$$

when M is the mEq of NaOH required for in the titration to end point. C is the percentage of ash remained after ignition. The reported D.S. values are means of three determinations (Kittipongpatana, Sirithunyalug, & Laenger, 2006).

2.4. FT-IR spectroscopy

FT-IR spectra were recorded on a Nicolet 510 FT-IR (Nicolet Instrument Corp., USA) using KBr disc technique. Spectra were baseline-corrected using Omnic ver.6.2 software and major peaks and bands were evaluated, in % *T* mode, as a confirmation for carboxymethylation.

2.5. Amylose and moisture contents

The amylose content (AC) of native and modified rice starches was determined according to the method described by Juliano (1971). The moisture content (MC) was carried out using a moisture balance (Kett, USA, model F-IA).

2.6. Physicochemical property and pharmaceutical functionality evaluations

2.6.1. SEM analysis

Scanning Electron Microscopy (SEM) experiments to analyze the granule surface, shape and size were conducted using a JEOL instrument model JSM-5910LV (JEOL, USA) equipped with a large field detector. The acceleration voltage was 15 kV under low vacuum mode (0.7–0.8 torr). The sample was placed on a copper stub covered with carbon tape and coated with gold. The magnification was set at 2000X.

2.6.2. X-ray diffraction

XRD patterns were recorded in the reflection mode on a Siemens D-500 X-ray diffractometer. Diffractograms were registered at Bragg Angle (2θ) = 5–40° at a scan rate of 2.5° per minute.

2.6.3. Thermal properties

Thermal properties were assessed using a Perkin Elmer DSC-7 differential scanning calorimeter. The analysis was carried out at a temperature between 30 and 120 °C, at 10 °C/min, on a 1:3 (w/w) starch–water mixture sample. An empty pan was used as a reference. The temperatures of the characteristic transitions, onset (T_o), peak (T_p) and conclusion (T_c) temperatures were recorded and the temperature ranges (T_c–T_o, ΔT) were calculated. Enthalpy (ΔH) was calculated and expressed as J/g of dry starch.

2.6.4. Swelling volume and water solubility

The swelling volume and solubility in water were determined by adding 0.1 g of sample into a pre-weighed centrifuge tube containing 10 mL water (1% w/v), mixed thoroughly for 1 min using a Vortex mixer, allowed to stand for 12 h, and observed the solubilization and/or swelling. The tube was then centrifuged at 3000 rpm for 15 min. The supernatant was transferred into a pre-weighed crucible and dried to a constant weight at 120 °C. The volume of the sedimented paste and the weights of the dried residue were used to calculate the swelling volume (mL/g) and the solubility percentage, respectively.

2.6.5. Free-swelling capacity

Free swelling capacity (FSC) was determined by using the teabag method described by Heß et al. (2007), with slight modification. Sample (0.8 g) was accurately weighed into a pre-weighed, dry teabag. The bag was tied and submerged in a beaker containing excess amount of water at room temperature. After 1, 5, 15, 30 and 60 min, the bag was taken out, the excess water was wiped out until no visible droplets were observed. The weight of the teabag and the content was determined. FSC value (q) was calculated as;

$$Q_{\text{FSC}} = \frac{m_t - m_{tb} - m_w}{m_s}$$