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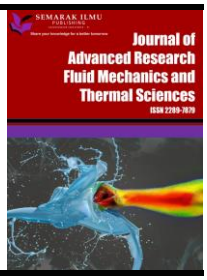

Chemical Surface Modification of Cornstarch Microparticles by Acetic Acid for Curcumin Carrier

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ABSTRACT

Starch without further processing has hydrophilic properties, making it have mechanical properties, and poor dimensional stability, especially in moist or water-rich environments. Thus, efforts to modify starch using chemical processes to make hydrophobic starch have been developed. This study aimed to modify and characterize chemically modified corn starch microparticles by acetic acid and their application as curcumin carriers. Corn starch was chemically modified using various concentrations of acetic acid (50, 75, and 99%). In this study, analyses of physicochemical characteristics (such as using Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM)), hydrophobicity/swelling characteristics, loading effectiveness, and loading capacity were performed. Modified corn microparticles showed a surface free of pores. According to the FTIR findings, acetic acid treatment was effective in changing the chemical characteristics of the corn starch surface. Hydrophobicity, loading efficiency, and loading capacity increased with increasing concentration of glacial acetic acid used. This study demonstrates the ability of hydrophobic corn starch microparticles as a carrier for active ingredients such as curcumin. This study can support the current sustainably development goals (SDGs).

1. Introduction

Starch is one of the most abundant biopolymers in nature and is usually isolated from plants in the form of micro-scale granules. Globally, the main sources of starch are corn (82%), wheat (8%), potatoes (5%), and cassava (5%). Starch can be utilized directly in its natural form or further processed through several modification processes (i.e. physical, chemical, and mechanical) to obtain specific characteristics suitable for certain applications [1]. Since starch is environmentally friendly, starch microparticles are suggested as one of the promising biomaterials for new uses in food, cosmetics, pharmaceuticals as well as various composites. Further, due to its chemically harmless, starch has been used for many educational experiments and practicum [2,3]. Study on starch can support the current sustainably development goals (SDGs).

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Starch without further processing has hydrophilic properties. Thus, it has mechanical properties, and poor dimensional stability, especially in moist or water-rich environments [4]. To overcome these weaknesses, efforts to modify starch using chemical processes to make hydrophobic starch have been developed. Chemical processes were selected because of their non-destructive characteristics, the possible functionality of modified starch, and the enhancement or introduction of key properties [5].

By adding hydrophobic functional groups, such as through acetylation of starch, hydrophobic starch can be produced [6]. Acetic anhydride is typically used in alkaline circumstances to acetylate starch [7]. However, acetic anhydride is extremely corrosive, dangerous, expensive, and scarce despite its high reactivity. In fact, in several countries, acetic anhydride reagents have strict regulations recommended by the United Nations (UN) to prevent illegal drug synthesis [8]. Thus, safer and simpler to acquire reagents were used in the development of starch modification techniques, such as acetic acid [9-11]. In this research, acetic acid was used to replace acetic anhydride and increase the hydrophobicity of starch.

Enhancing starch's hydrophobicity can make it more compatible with hydrophobic materials and molecules [12]. The use of starch in food, cosmetics, composite materials, and medicine may be expanded as a result of these characteristics. Hydrophobic starch has excellent carrier ability, particularly for materials that are sensitive or active due to heat or light [13]. Additionally, the carrier of a starch-containing substance can improve the material's stability, relative humidity, and water solubility [14]. In this study, curcumin was used as a model of the hydrophobic active ingredient that would be incorporated into starch microparticles that had been prepared.

Curcumin is one of the main phenolic pigments, extracted from turmeric (rhizoma of Curcuma longa) along with demethoxy curcumin and bisdemethoxy curcumin [15]. Curcumin contains antioxidant, anti-inflammatory, anti-HIV, and anti-microbacterial abilities, to cure cancer [16]. Curcumin is naturally hydrophobic, so it is difficult to dissolve in water but dissolves in ethanol, dimethylsulfoxide, and acetone [17]. Curcumin is also highly light-sensitive despite being thermally stable [18]. This weakness can be overcome by using modified starch microparticles as curcumin carriers [19,20].

As most reactions require the material to be in solution or a slurry and the reagents frequently are too harsh for application, there are very few techniques that apply to preformed starch particles. The main novelty of this research was the preparation of hydrophobic preformed starch microparticles in a slurry at ambient room temperature with constant pressure and pH condition. The starch microparticles' compatibility with hydrophobic compounds (curcumin) was also assessed in this study using curcumin.

In addition, this study focuses on the analysis of acetylated starch particles at the micrometer scale. As opposed to nanoparticles, bulk, and film materials, micrometer-sized particles have their benefits. Microparticles can easily settle, different from nanoparticles which require special handling throughout the separation process. This settling ability also prevents microparticles from contaminating the sample during analysis, resulting in a precise and accurate assessment.

Based on our previous studies regarding cornstarch research [21-25]. This study aimed to modify and characterize chemically modified corn starch microparticles by acetic acid and their application as curcumin carriers. Corn starch was chemically modified using various concentrations of acetic acid (50, 75, and 99%). Physicochemical characteristics (i.e. Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM)), hydrophobicity/swelling characteristics, loading effectiveness, and loading capacity were analyzed. Results from this study can be used in optimizing and developing drug delivery designs from curcumin with hydrophobic starch molecules.

2. Methodology

2.1 Materials

The materials used in this study were corn starch (SASA, Indonesia) and turmeric powder (DESAKU, PT. Mutasa Indonesia). We also used acetic acid, distilled water, acetone, ethanol, NaOH, and HCl, which were purchased from the local markets in Bandung.

2.2 Hydrophobic Starch Microparticles Preparation

5.5 g of corn starch dissolved in 45 mL of distilled water. The solution was stirred for 15 minutes until a homogeneous suspension was formed. 1 M NaOH was added to the solution until pH = 8. Acetic acid with a certain concentration (50, 75, and 99%) of as much as 15 mL was added dropwise with constant stirring (1000 rpm; 60 minutes). The suspension was added with 6.6% HCl until pH = 5.5. After cooling to room temperature, the suspension was centrifuged (11000 rpm; 1 minute) to obtain a white solid. The solid was rinsed with cold ethanol 2 times and centrifuged several times (11000 rpm; 1 minute). The solids are then dried in an oven (50°C). The labels of HS-050, HS-075, and HS-099 are then used in this article to refer to the concentration of acetic acid used in the sample treatment.

2.3 Physicochemical Characteristics of Hydrophobic Starch Microparticles

The physicochemical characteristics were analyzed using SEM and FTIR. For FTIR analysis, the synthesized material is mixed with KBr with a ratio of 1:99. The sample and KBr mixture were crushed until the sample and KBr were evenly mixed. The crushed mixture is placed in a pellet mold and pressed with a hydraulic press to form pellets. The pellet was then placed in the holder and characterized with the FTIR spectrophotometer at wave numbers 4000-400 cm^{-1}. The FTIR spectrophotometer instrument used was the FTIR-600, Jaco Corp, Japan.

SEM characterization was carried out using a Carl Zeis Type EVO MA 10. The sample to be analyzed was attached to the specimen holder. Samples that have been mounted on the holder are cleaned using a hand blower. The sample is then put into the coating machine to be given a thin layer of gold-palladium for four minutes to produce a layer with a thickness of 200-400Å. The coated sample is then put into the sample chamber. SEM images were taken at x500 and x2500 magnifications.

2.4 Swelling Capacity

A sample of 500 mg of hydrophobic starch (W_0) and 25 mL of water were added to the vial. The mixture was then stirred and heated at 60°C for 30 minutes. After that, the mixture was cooled to room temperature and centrifuged (11000 rpm; 1 minute).

The starch that has undergone swelling is then separated from the supernatant and weighed. The residue (along with the water contained in the starch) denoted as W_r is then weighed. Eq. (1) is used to calculate the swelling capacity (*SWP*)

$$\mathrm{SWP}\ (\%) = \frac{\mathrm{W_r}}{\mathrm{W_0}} \times 100 \tag{1}$$

2.5 Loading Curcumin with Hydrophobic Starch Microparticles

0.5 g of hydrophobic starch was dissolved in 30 mL of acetone and stirred for 15 minutes (1000 rpm). 30 mL of distilled water was added and then stirred for 20 minutes. The mixture was centrifuged (11000 rpm; 1 minute) and 1 mL of supernatant was taken for loading efficiency test. The mixture was again stirred for 20 minutes and the supernatant was taken after the centrifugation process. This step is done every 20 minutes until the 80th minute.

After centrifugation, the solids were taken and washed twice with ethanol until the filtrate was clear and then were dried in an oven (50°C). The modified corn starch particles that were loaded with curcumin were then weighed as much as 200 mg, mixed with 30 mL of water, and stirred for 15 minutes (1000 rpm). After that, the mixture was centrifuged and the supernatant was tested for maximum absorbance at a wavelength of 422 nm using a UV-VIS instrument. Curcumin solutions were prepared and tested similarly.

2.6 Loading Capacity and Loading Efficiency

1 mL of the supernatant from the previous step was diluted with 9 mL of distilled water and the absorbance was measured at 422 nm using a UV-VIS spectrophotometer (Model 7205; JENWAY; Cole-Parner; US). Curcumin concentrations were calculated using standard curcumin solution series curves. Loading efficiency is measured every 20 minutes until the 80th minute. The loading efficiency *(%LE*) and loading capacity (*LC*) of curcumin in hydrophobic starch were calculated using Eq. (2) and Eq. (3), respectively.

$$\%LE = \frac{W_{EC}}{W_C} \times 100 \tag{2}$$

$$LC = \frac{W_{EC}}{W_{MP}} \tag{3}$$

where *LE* is the loading efficiency (%), *LC* is the loading capacity (%), W_{EC} is the mass of loaded curcumin (mg), *Wc* is the total mass of curcumin used during the test (mg), and W_{MP} is the mass of microparticles (mg).

3. Results

3.1 SEM and FTIR Analysis

Figure 1 shows the SEM photos of the prepared hydrophobic starch. Detailed information for the analysis of SEM is reported in our previous study [26]. Treatment with acetic acid did not change the morphology and surface topology of the corn starch microparticles. Acetic acid is a weak acid. Stronger acids like HCl and H_2SO_4 can be used to treat starch to alter its structure and morphology [27]. Acetic acid modification is effective for getting corn starch with a minimal surface change. The prepared microparticles were found to be 9-18 µm in size.

Figure 2 shows the results of the FTIR analysis of prepared hydrophobic starch. Detailed information on how to read and interpret FTIR is shown in our previous studies [28-31]. In corn starch, the peak at 1656-1640 cm^{-1} is associated with the angular bending of the O-H groups of water molecules, the peak at 2960 cm^{-1} is associated with C-H vibrations, and the wide band at 3450 cm^{-1} indicates vibration of the hydroxyl groups. The absorption band at 1116 cm^{-1} is associated with the -

C-O- stretching on the polysaccharide skeleton [26]. This is in line with our previous studies regarding cornstarch [21-25].

Treatment with acetic acid did not cause an acetylation reaction. There was no increase in the peak height and area at the bands 1250 and 1740 cm^{-1} and no decrease in the band at 1645 cm^{-1} that showed acetylation. However, decreases in the intensity peaks of corn starch have been observed due to the treatment. Modification occurred as a result of acetic acid's interaction with starch's functional group. Apart from using FTIR, the presence of acetyl in the corn starch backbone can be identified by the titration method [32].

The mechanism of reaction between acetic acid and starch was shown in Figure 3. Here, in line with earlier investigations [33], this study also used NaOH as a catalyst for the acetylation reaction. However, because the acetyl peak was not found in FTIR analysis, this reagent was not particularly favorable for the acetylation reaction between starch and acetic acid.

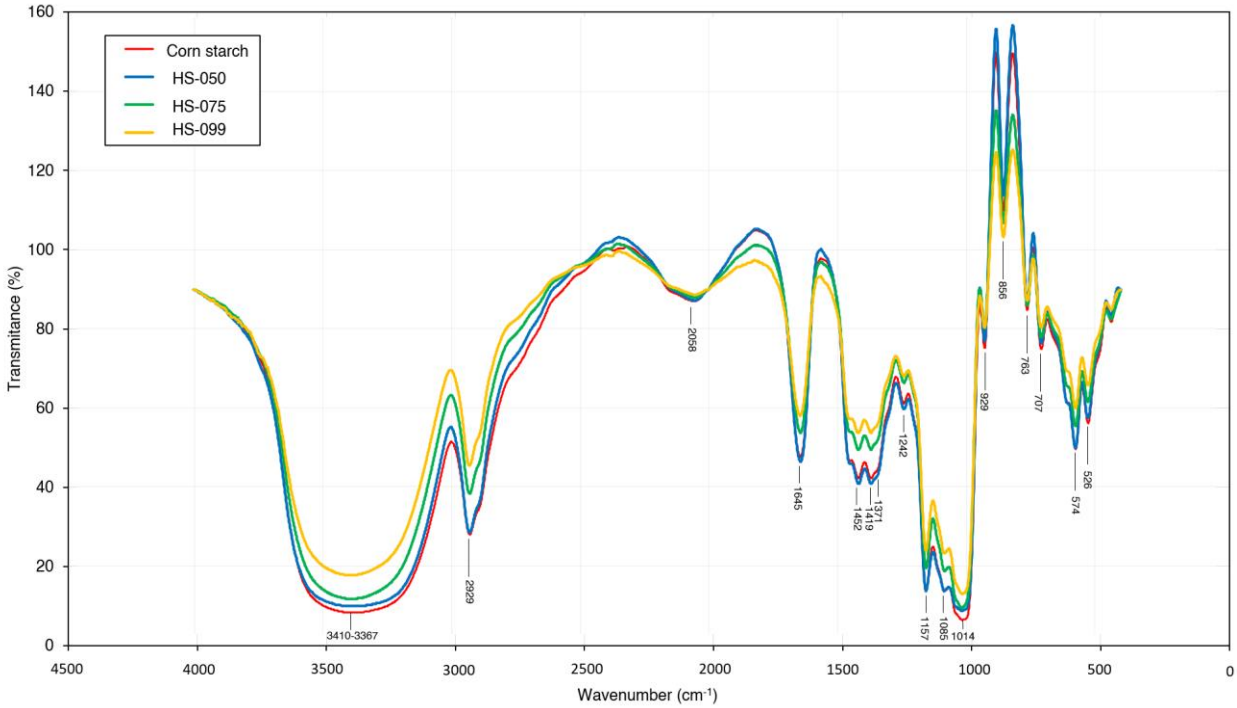

Fig. 1. FTIR results of prepared hydrophobic starch microparticles

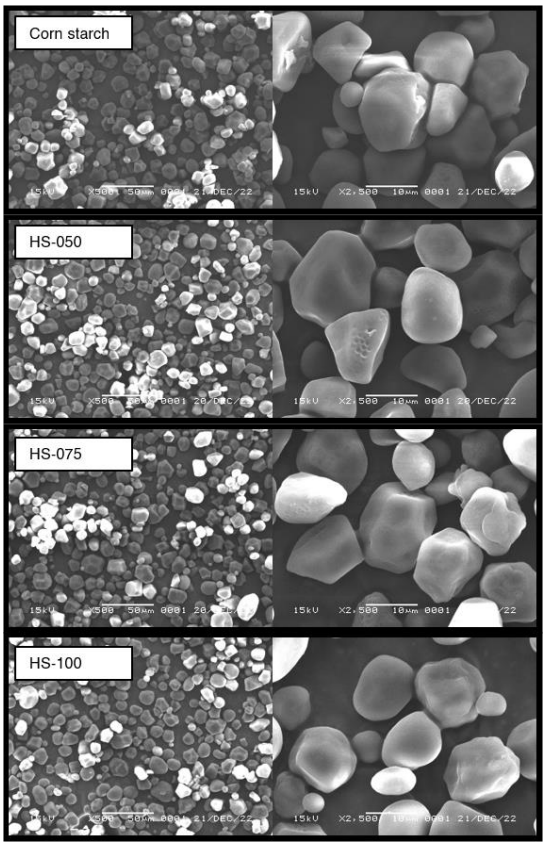

Fig. 2. SEM results of the prepared hydrophobic starch microparticles

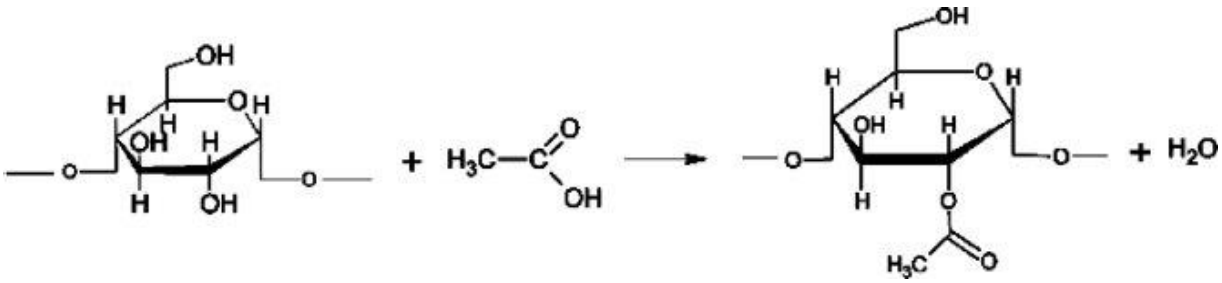

Fig. 3. Acetylation reaction between acetic acid and starch

3.2 Swelling and Hydrophobicity

Figure 4 shows the swelling percentage of prepared hydrophobic starch. Starch consists of amylose and amylopectin chains. This long and complex starch structure causes it to be difficult to dissolve in water at room temperature. Starch granules can integrate with water at high temperatures. When starch is heated in excess water, the intermolecular bonds in the starch are broken. This causes the hydroxyl sites to interact with water and cause swelling. The degree of swelling indicates the capacity of starch to hold/absorb water. Factors that can affect the swelling power, solubility, and water-binding capacity of starch are

i. opening the starch structure at a low degree of substitution, so that water access into the starch structure becomes easier,
ii. increased hydrophobicity of the polymer chain due to acetyl groups.

Corn starch without any modification has the highest swelling level which is around 31.28%. Modification of corn starch with acetic acid causes a decrease in the percentage of swelling. This trend is mainly found in starch with a high degree of substitution.

At a sufficiently high degree of substitution, the hydroxyl groups in starch are replaced by acetyl groups. As a result, fewer hydroxyl groups can interact with water. Thus, the swelling power decreases. The trend of decreasing swelling power due to the high degree of substitution was also reported in the acetylation of cassava starch, pea, sago, and rice.

Acetic acid was assumed to have succeeded in modifying the starch surface, resulting in blocking the water access to the starch structure. The decreases in swelling power also can be attributed to the disruption of amylopectin's side chains, and increases in soluble dextrins in starch granules.

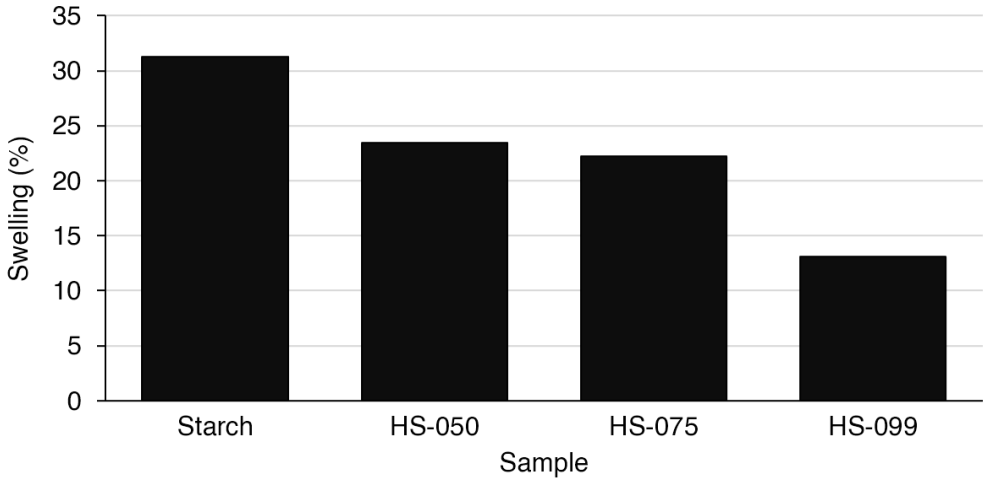

Fig. 4. Swelling capacity of prepared hydrophobic starch

3.3 Loading of Curcumin with Hydrophobic Starch

Table 1 shows the loading capacity and maximum UV-VIS absorbance. The loading capacity increases with increasing concentrations of acetic acid used. The highest loading capacity was found in the starch treatment with an acetic acid concentration of 99%, namely 52.26%. The maximum absorbance of prepared hydrophobic starch with loaded curcumin was also tested (Table 1). The maximum absorbance value decreases with the increasing concentration of glacial acetic acid used. The low maximum absorbance indicates the success of starch as a carrier agent. In addition, the hydrophobic nature decreases the solubility of curcumin in water so that the maximum absorbance value is low.

Figure 5 shows the loading efficiency (%LE) of prepared hydrophobic starch microparticles. Loading efficiency gradually increases with loading time. The highest % LE was obtained in the HS-099 sample with a value of 73.54% with a loading time of 80 minutes. %LE increases with increasing concentration of glacial acetic acid used. Modification of starch with acetic acid succeeded in increasing the %LE of loading curcumin in corn starch.

This finding was higher than the %LE observed in loading curcumin on cassava starch nanoparticles (66.57%) [32]. The %LE value obtained is lower when compared to the results of acetylation of banana starch microparticles using acetic anhydride reagent which provides a loading efficiency of 85-90% [34]. These findings demonstrated that, despite nanoparticles having larger surface areas than microparticles, modified microparticles were comparably effective at loading curcumin. A visual representation of the curcumin release from the modified starch was shown in Figure 6.

Table 1
Loading capacity and UV-VIS maximum absorbance (λ = 422 nm) of prepared hydrophobic starch loaded with curcumin

Sample	Loading capacity (%)	Maximum absorbance (λ = 422 nm)
HS-050	34.43	0.089
HS-075	43.02	0.058
HS-099	52.26	0.034
Curcumin solution	-	0.492

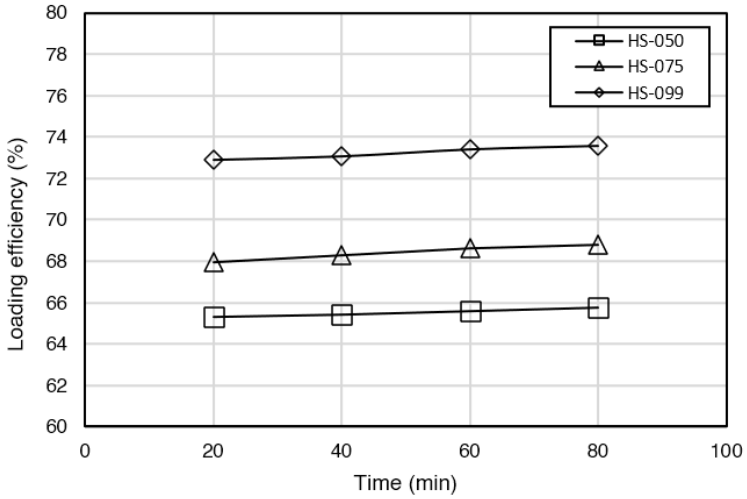

Fig. 5. Loading efficiency of prepared hydrophobic corn starch microparticles

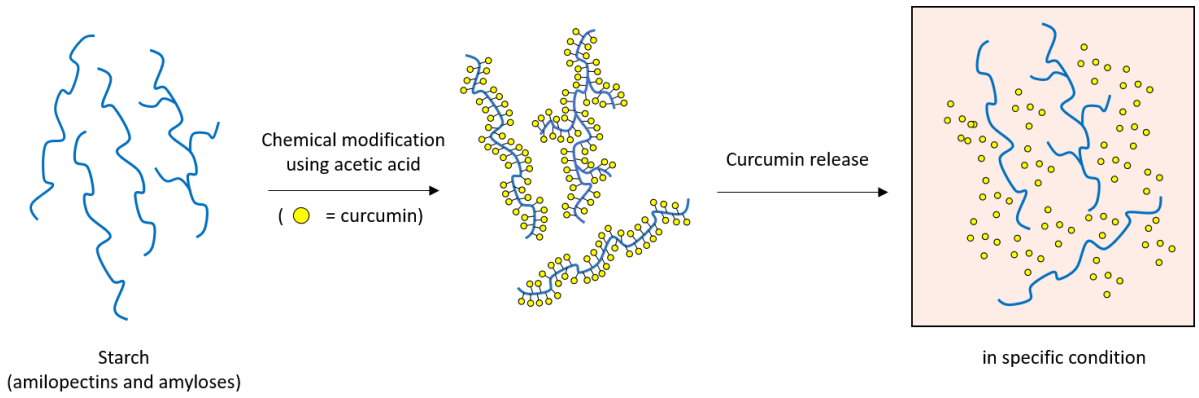

Fig. 6. Visual representation of the curcumin release from the modified starch

4. Conclusions

Corn starch microparticles were chemically modified using various concentrations of acetic acid (50, 75, and 99%). Hydrophobicity/swelling, loading efficiency, and loading capacity of hydrophobic starch increased with increasing concentrations of glacial acetic acid used in the acetylation process. The highest loading efficiency for the HS-099 sample was 73.54% with an optimal loading time of 80 minutes. Modification of corn starch with acetic acid causes a decrease in swelling power due to the hydrophobic acetyl group. This study demonstrates the ability of hydrophobic corn microparticles as a carrier for hydrophobic molecules such as curcumin.

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