Effect of heat treatment and acid hydrolysis on microstructure of corn starch at below gelatinization temperature

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Abstract
Starch in its natural form was not suitable for using in some applications due to its insolubility in water and low stability and needs to be modified. In present study, the heat treatment and acid hydrolysis of corn starch was performed at 60, 65 and 70 °C with the pH of 2, 3 and 4 for 30 and 45 min. The decreasing of pH to 2 and increasing the heating duration time to 45 min had significant effect on increasing the amylose and reducing sugar concentrations. However, the temperature showed no significant effect in range of 60 to 70 °C on the starch hydrolysis. Therefore, maximum increasing in amylose (0.13 g/g starch) and reducing sugar (0.15 mg/g starch) concentrations were obtained at 70 °C with the pH of 2 for 45 min.

Keywords: Starch, Heat treatment, Acid hydrolysis.

Introduction
Starch is one of the most widely used biomaterials in various industries such as food, pharmaceutical and health due to its unique properties such as biocompatibility and renewability [1]. Starch structure consist of linear glucose chain called amylose and branched glucose chain called amylopectin. Some of starch characteristics like insolubility in water and gelatinization restrict its potential application in some industries [2]. Starch particles are widely used in drug delivery systems as binders and fillers. However, native starch in its natural form are not suitable for using in targeted drug delivery systems due to limitations such as rapid release of drug in physiological fluids and low solubility in water. To achieve better physical, chemical and functional properties, the starch granules should be modified. The starch modification rules divided in three groups of physical, chemical and enzymatic methods [3-5]. Moister, heat, shear or radiation are used in physical modification rules to improve the starch properties and these methods became popular due to the lack of chemical wastes. Heat treatment is one of the prevalent physical methods that changes the physiochemical characteristics of starch. Kong et al. incubated corn starch suspension with the concentration of 45% w/w at 60, 62.5 and 65 °C for 15 min. They reported that during the heating process the starch granules swelled and many pores and pits were observed on the starch granules structure which would facilitate the physical access to the interior parts of the granules [6].

When native starch is heated in excess water, the granules started to swell and loose their crystallinity. After reaching the maximum swelling rate, the granules will burst and the amylose molecules release in the medium. The gelatinization temperature depends on the starch source and occurs between 60-70 °C [2, 7]. The gelatinization temperature of corn starch were reported 70 °C by Li et al. [8]. In this process, the starch solution viscosity increases and consume a lot of energy. So the industry process prefer to perform the heat treatment below the gelatinization temperature [8].

The starch chemical modification methods involve reaction that introduce new functional groups or fracture the starch structure. One of the most useful chemical modification method is acid hydrolysis. In this process that essentially performs below the gelatinization temperature by mineral acids like hydrochloric acid, sulfuric acid and nitric acid, the amorphous regions are hydrolyzed and the amylose molecules release from the starch structure. In addition, some of the released amylose converts to reducing sugar molecules like glucose and dextran. So the amylose and reducing sugar concentration increase [3]. In the study of Li et al. acid hydrolysis of potato starch using HCl (1M) at 40 °C for 5 h was performed. They reported that in the process lots of large and irregular cavities were observed in starch structure [9].

Therefore, to increase the application of starch in some industries such as drug delivery processes, their solubility and stability in biological environments can be improved simultaneously by using heat treatment and acid hydrolysis methods. In present research, the effect of heat treatment and acid hydrolysis on corn starch granules were studied. The starch heating temperature, solution pH and heating duration time were considered as parameters and the amylose and reducing sugar concentrations were measured as responses to examine the efficiency of the present method and determine the optimal conditions.

Experimental

Materials

Corn starch were bought from Sahra company and hydrochloric acid from Merck Company. Double distilled water was used for preparing the suspension.

Heat treatment and acid hydrolysis procedure

This process was performed at a concentration of 5% (w/v) of native corn starch at three temperatures of 60, 65 and 70 °C with adjusted pH equal to 2, 3 and 4 for 30 and 45 min. First, the corn starch solution with the concentration of 5% (w/v) and different pHs of 2, 3 and 4 adjusted with hydrochloric acid was prepared. Then the solution was heated for 30 or 45 min in the water bath at 60, 65 and 70 °C with the rotation speed of 500 rpm. Then the amount of amylose and reducing sugar concentrations were measured. The study was performed by general factorial design method.

Analysis

The concentration of amylose that released in the medium during the hydrolysis process was measured by Mcgrance et al. and Xiao et al. methods [10, 11].

The reducing sugar concentration that produce during starch hydrolysis process were determined by Miller method [12].

Results and discussion

The amounts of changing of amylose and reducing sugar concentrations after heat treatment and acid hydrolysis processes are reported in Figs. 1 and 2.

Effect of factors on amylose concentration

During heat treatment and acid hydrolysis some of linear part of starch particles were released in medium so the amylose concentration was increased . As shown in the diagrams of Fig. 1, the changes in amylose concentration at all three pHs of 2, 3 and 4 and at all three temperatures of 60, 65, and 70 °C were increased over time. The highest increase in amylose concentration was obtained in 45 minutes. It is shown that with increasing hydrolysis time, more amyloses are released in the reaction medium.

In addition, the amylose concentration increased at a constant temperature with decreasing pH. The highest rate of changes in the amylose concentration was observed at pH of 2, which can be concluded that acid hydrolysis at low pHs had a more effective result in breaking down starch particles and releasing amyloses. However, considering a constant pH, by increasing temperature, increasing changes in the released amylose concentration did not have a logical trend (P-value=0.0676). The ineffectiveness of temperature on amylose concentration changes may be due to the proximity of temperature levels.

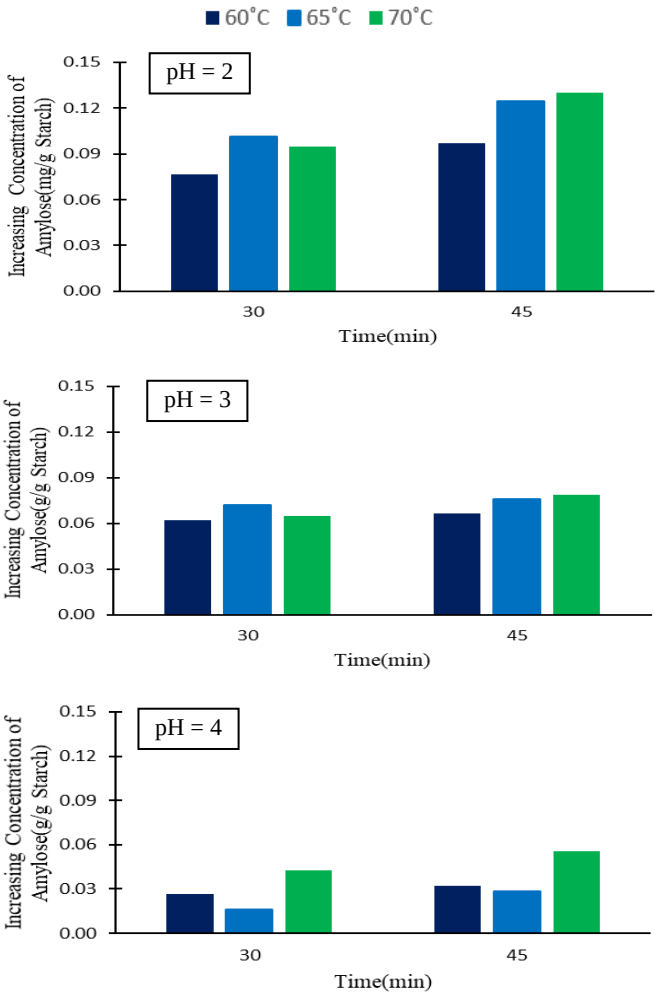

Fig. 1 Experimental determination of Amylose concentration versus time at 60, 65 and 70 °C and various pHs

Therefore, it can be concluded that if the experiment is performed at an initial pH of 2 and heated for 45 minutes at 70 °C, the maximum incremental changes in the released amylose concentration can be achieved equal to 0.13 (g/g starch). In the research of Li et al., the heat treatment of corn strach was performed and the optimum temperature of acid hydrolysis was reported to be 67.5 °C [8].

Effect of factors on reducing sugar concentration

In the starch hydrolysis process, part of the released amylose is converted to reducing sugar so the reducing sugar concentration were increased. However, most of the amylose structure remains intact, so the concentration of amylose increased.

As shown in the diagrams of Fig. 2, the increasing changes in the reducing sugar concentration at all three pHs of 2, 3, and 4 at all three temperatures of 60, 65, and 70 °C were increased almost over time and the most changes in the reduction sugar concentration being achieved in 45 minutes. It can be concluded that heat treatment and acid hydrolysis over time resulted in low hydrolysis of free amylose and their conversion to reducing sugar. The result is in agreement with Kong et. al study that the reducing sugar concentration were increased by heating corn starch suspension (45%, dry basis, w/w) at 60 °C for 15 minutes [6].

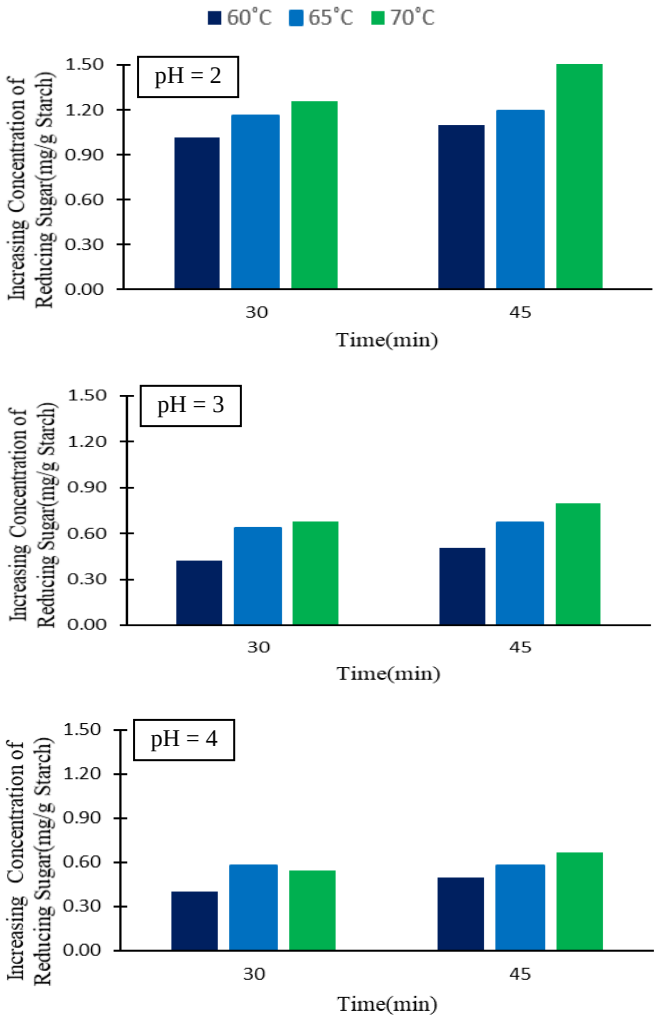

Fig. 2 Experimental determination of reducing sugar concentration versus time at 60, 65 and 70 ° C and various pHs

In addition, the increasing changes in the reducing sugar concentration at a constant temperature with decreasing pH had an increasing trend and at pH of 2 the most changes in the reducing sugar concentration were observed, which can be concluded that acidic hydrolysis at low pHs has been more effective in hydrolyzing starch particles and converting them to reducing sugar. While at a constant pH with increasing temperature, increasing changes in reducing sugar concentration did not have a logical trend (P-value=0.0584). The changes in heating tempreture due to proximity of temperature levels didn't have a significant effect on increasing reducing sugar concentration. Therefore, it can be concluded that if the experiment is performed at 70 °C, pH of 2 for 45 minutes, the most increasing changes in reducing sugar concentration equal to 0.15 (mg/g starch) was obtained. So if the experiment was performed at 70 °C with pH of 2 for 45 min the maximum yield in increasing the amylose and reducing sugar concentration equal to 0.13 (g/g starch) and 0.15 (mg/g starch), respectively were obtained. Performing the study at this temperature range was suitable because the starch granules was not gelatinized and the solution viscosity didn't change.

To achieve higher efficiency in the hydrolysis of starch particles and increase the concentration of reducing sugar, a heat treatment and acid hydrolysis duration can be increased. Saguilan et al. performed acid hydrolysis of native banana starch with HCl 1M at 35 °C for 6 h in water bath. They reported that the increasing in amylose concentration was 37% [13]. In the study of Okunlola et al. the native *Dioscorea* starch were hydrolyzed with HCl solution 6% (v/v) at 23 °C in 192 h for using as a binder chloroquine phosphate tablets. They reported that the remaining amylose content in modified starch was decreasing equal to 15.33 % because some of the amylose molecules were released in the medium during the hydrolysis [14]. In the research of Nasrin et al., who hydrolyzed corn starch with HCl acid for 45 minutes, it was reported that acid hydrolysis was not effective in increasing the concentration of amylose and reducing the starch hardness due to the short time of acid hydrolysis process [15].

Optical microscope images of modified starch with concentration of 0.25 % (w/v) and pH of 4 at temperatures of 60, 65 and 70 °C are shown in Fig 3. In this process, due to the low temperatures of 60 and 65 °C, the starch particles had a sedimentary state but at a temperature of 70 °C, the starch particles had a slightly gel state. In the research of Li et al., the onset temperature of gelatinization of corn starch was reported 70 °C [8] .

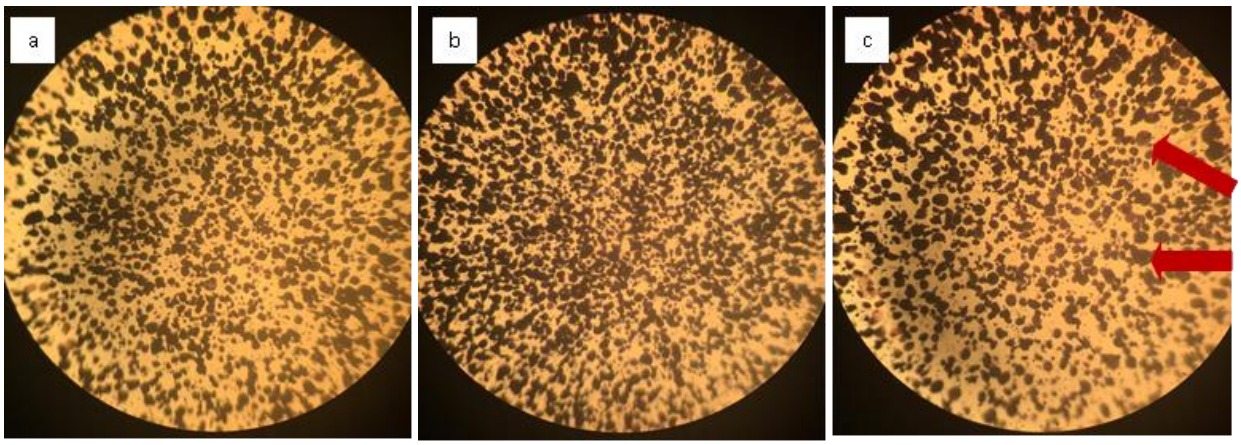

Fig. 3 Optical microscope images of starch with a concentration of 0.25%(w/v) after acid hydrolysis at pH=4 and heat treatment at temperature of a) 60, b) 65 and C) 70 °C (pointed gelatinized starch granules)

Conclusions

The starch particles due to its insolubility in water and low stability are not suitable for using in drug delivery process as binders and fillers or food and pharmaceutical industries. For improving its physicochemical properties, the starch particles should be modified. In present study heat treatment and acid hydrolysis of corn starch was performed at 60, 65 and 70 °C and pH of 2, 3 and 4 for 30 and 45 min. During the process the reducing sugar and amylose concentration were increased with increasing the heat duration time to 45 min and decreasing the pH to 2 .Temperature changes was not significant due to the small considered temperature range. Therefore, if the experiment was performed at 70 °C with pH of 2 for 45 min the maximum efficiency in increasing the amylose and reducing sugar concentration were obtained equal to 0.13 (g/g starch) and 0.15 (mg/g starch), respectively. To achieve higher efficiency in braking down the starch particles and producing reducing sugar, the heating treatment and acid hydrolysis duration time can be increased.

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