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# Studies on the optimisation of preparation of succinate derivatives from corn and amaranth starches

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## Abstract

Conditions for the preparation of half esters of corn and small sized (1–2 μm) waxy amaranth starch using succinic anhydride were compared in a non-aqueous medium. The synthesis was followed in terms of % succinyl content and the degree of substitution (DS). The parameters optimised included succinic anhydride concentration, reaction time and starch/pyridine ratio required in the process. Succinate derivatives from both the starches gave DS as a function of the succinic anhydride concentration and reaction time. A starch/pyridine ratio of 1:2 was found to be necessary for the reaction to be carried out smoothly. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords*: Corn starch; Amaranth starch; Succinate derivatives

## 1. Introduction

Derivatisation of starch with an ionic substituent group such as succinate, at low degree of substitution (DS) converts it into a polyelectrolyte. Starch then acquires typical properties of a polyelectrolyte such as increased water solubility (Robert, 1967) and increased solution viscosity. These charged macromolecules are attracted by opposite charge, and consequently they find use in paper industry as thickener, flocculants and strengthening agent (Oppermann, Nebel, Dorn & Buchholz, 1996; Homa, Koenig & Haung, 1994). Besides pyridine, organic bases such as α-picoline, pyrrole, triethylamine pipridine have also been reported as catalyst for esterification of starch (Robert, 1965, 1967). Starch granules kept in contact with acid anhydride in pyridine at room temperature for five months reportedly showed no noticeable reaction (Mullen & Pascu, 1942). Hence, starch granule must be disrupted appropriately for succinylation to occur.

Low DS starch succintes could be obtained by refluxing it in pyridine at 115°C in the presence of succinic anhydride for varying reaction time without prior gelatinisation (Lohmar & Rist, 1950; Rutenberg & Solarek, 1984; Billmers & Tessler, 1995). Another method of preparation involved gelatinisation of starch by heating it in 60% aqueous pyridine, distilling the pyridine water azeotrope (bp 93°C) from the mixture, and replacing the distillate with 100% pyridine as the azeotrope was removed. When last traces of azeotrope was removed, the temperature reached 115°C. At this point acid anhydride was added and the reaction reached completion in about 5–10 min (Lohmar & Rist, 1950; Mullen & Pascu, 1942).

Differences in the reactivity of starches appears to be related to the granule size. Starches of larger granule size are less reactive (Trubiano, 1987). The pyridine treatment does not gelatinize the starch, the granules being birefringent while still swollen. The activation of starch was most important, both in determining the reactivity and the solubilities of the starch succinates prepared therefrom (Wolff, Old & Hilbert, 1946). Starch succinate with high DS can also be prepared by glacial acetic acid containing sodium acetate at 100°C (Mc.Intire, 1958). Recently, extrusion has also been employed to prepare starch succinates using succinic anhydride (Tomasik, Wang & Jane, 1995). Starch succinates show improved properties like decreased gelatinisation temperature, improved freeze-thaw stability, viscosity stability and better clarity (Trubiano, 1987).

The mechanism of base catalysed reaction of pyridine with starch is considered to be a nucleophilic substitution ($SN^2$) reaction and passes through the formation of an intermediate complex. The substitution takes place mainly by an addition–elimination mechanism (Whistler & Bemiller, 1959; Bunton, 1963). Pyridine serves to increase the initial reactivity of the granules and also works as a catalyst for the reaction through the formation of the succinyl-pyridinum intermediate. The reaction proceeds as follows.

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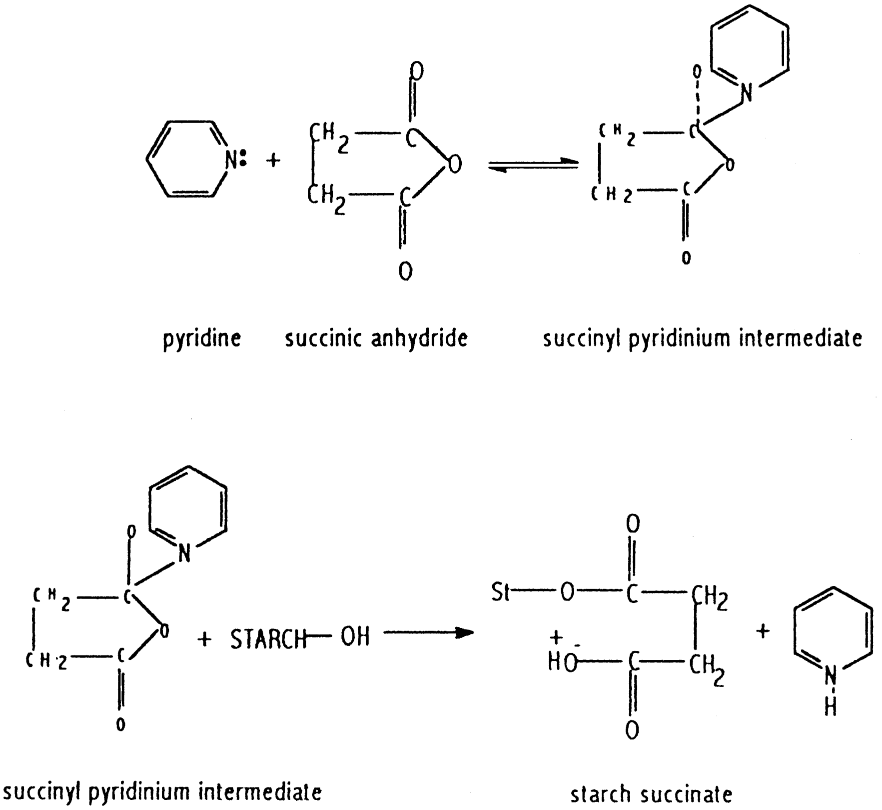

In the substitution reaction, the starch molecule gets activated first and becomes nucleophilic due to an activating catalyst pyridine. Succinic anhydride reacts with pyridine to form succinyl-pyridinum intermediate. This is followed by collision of starch–O to the carboxyl group of succinic anhydride where pyridine is attached. The hydroxyl oxygen of starch has more electronegativity, and hence has a tendency to link with carboxyl group. This results in the elimination of pyridine. Moreover, the efficiency of the reaction is influenced by the reagents used. Reports on the manufacture of starch succinates are scanty. However, Mullen and Pascu (1943) prepared starch succinates from corn starch. But these workers concentrated on the role of pyridine and other catalysts in the manufacture of starch succinates. Very few reports on the process parameters for succinylation of starches are available (Tessler & Wurzburg, 1983; Jarowenko, 1987; Zou & Chen, 1992).

The US FDA permits reaction of the starch upto 4% succinic anhydride for food applications (Trubiano, 1987). The present work is a systematic approach to prepare low DS starch succinates (<0.20) from corn starch. Amaranth starch isolated in the laboratory from *Amaranthus paniculatus* grains was chosen as a model starch to see the effect of starch characteristics, if any, on the succinylation process, due to its extremely small granule size (1–2 μ) acid) and the absence of the linear amylose polymer. The process was optimised using anhydrous pyridine as a catalyst.

## 2. Materials and methods

### *2.1. Materials*

Corn starch obtained from M/S Raptakos Brothers, Thane and 60 mesh amaranth starch isolated in the laboratory by alkali steeping method (Yanez & Walker, 1986) from *A. paniculatus* Linn (Rajgeera) was used in the present work. All other reagents used in this work were of analytical grade.

### *2.2. Methods*

#### *2.2.1. Effect of process parameters on succinylation of the starches*

Esterification was carried out by taking the accurate amount of starch and the etherifying agent, succinic anhydride in a three neck 250 ml round bottom flask fitted with a mechanical stirrer. A heating mantle was used to control the reaction temperature. The overhead stirrer was fitted for uniform and constant stirring throughout the reaction time. The liquid catalyst, pyridine was added through one of the necks using a funnel. The other neck was fitted with reflux condenser attached with calcium chloride drying tube. The third neck was fitted with the thermometer pocket. The reaction was carried out by refluxing pyridine for varying time periods at 115°C to get the starch succinate. The reaction vessel was then cooled to room temperature. Starch succinate was isolated from the reaction mixture of pyridine by precipitation in absolute ethanol. The product was washed 3–4 times successively with ethanol to remove the traces of pyridine from the product. It was then dried in a vacuum desiccator over calcium chloride and finally in an oven at 50°C for 5–6 h (Wolff et al., 1946; Lohmar & Rist, 1950; Trubiano, 1987). Traces of pyridine that may remain in the product can be removed by adding calculated amount of dilute hydrochloric acid. This neutralizes the pyridine by forming a soluble salt that can be separated by vacuum filtration. The efficacy of removal of pyridine was checked by measuring the pH of the separated solution and ensuring it to be neutral (Mullen & Pascu, 1946).

The process parameters like succinic anhydride concentration, starch/pyridine ratio, and the reaction time were varied and optimised for getting the starch succinates form both corn and amaranth starches.

### *2.2.2. Determination of DS of corn and amaranth starch succinates*

The percent succinyl content of the succinate derivatives prepared as described above was determined by alkali saponification followed by titration of the excess alkali (Genung & Mallatt, 1941). The samples were dried in oven prior to analysis.

For the estimation of DS of the corn starch succinates (CSS) and amaranth starch succintes (ASS), 1 g of finely ground sample was weighed accurately and placed in a 250 ml Erlenmeyer flask or conical flask. Fifty millilitres of 75% ethanol in distilled water was added and the flask was loosely stoppered. The contents were warmed to 50°C while stirring and held at this temperature for 30 min. The flask was cooled to room temperature after which 40 ml of 0.5 N NaOH was added while swirling. The flask was stoppered and allowed to stand for 72 h at room temperature with occasional swirling. The sodium hydroxide solution saponifies the starch and excess alkali remaining in the medium was titrated with 0.5 N hydrochloric acid using phenolphthalein indicator. Additional alkali that might leach from the sample is titrated after the mixture is permitted to stand for an additional 2 h. Some times the product may undergo minor degradation because of treatment with caustic in alcoholic system. Therefore it is necessary to treat the native starch in the same manner to obtain the blank value. The percentages of succinyl and the DS of the sample were calculated by using the formulae given below (Mullen & Pascu, 1946; Wolff et al., 1946; Genung & Russel, 1941; Murry, Staud & Gray, 1931; Robert, 1967).

$$\%\ \text{succinyl} = \frac{(\text{Blank titre} - \text{Sample titre}) \times 0.1 \times \text{Normality of acid} \times 100}{\text{Weight of the sample taken (g)}}$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \%\ \text{succinyl}}{10000 - (99 \times\ \%\ \text{succinyl})}$$

or

$$\text{Degree of substitution (DS)} = \frac{162 \times (\%\ \text{succinyl}/100}{100 - (99/100 \times \%\ \text{succinyl})}$$

## 3. Results and discussion

Generally, low DS starches are recommended for food applications. Therefore, an attempt was made to study the preparation of starch succinates with varying levels of modification up to 0.20 DS and by varying the process parameters for both corn and amaranth starches. The parameters chosen for optimisation were reaction time, concentration of succinic anhydride, and pyridine/starch ratio. The optimisation process was started by varying two parameters at a time and keeping other constant. Initially the effect of reaction time on the % succinyl content and the DS was observed on addition of succinic anhydride (1–3%) for both the starches as shown in Figs. 1–4. The reaction time varied randomly from 1 to 5 h (Wolff et al., 1946) using 1:2 starch/pyridine ratio (Mullen & Pascu, 1941; Khalil, Hashem & Hebeish, 1995; Gerhard & Ute, 1996). The starch succinates so obtained were calculated for % succinyl content and DS. % Succinyl content was estimated saponifying the samples in ethanolic medium followed by back titration of the excess alkali with that of 0.5N hydrochloric acid. Some researchers used methanol instead of ethanol as a solvent during the saponification and reduced the time of analysis of % succinyl from 72 to 12 h (Mullen & Pascu, 1946). Malm, Genung, Williams and Pile (1944) used mixture of pyridine and methanol with methanolic potassium hydroxide in the presence of small amount of water for saponification purpose. Results from Fig. 1 clearly indicates that % succinyl content increased from 0.99 to 6.13% with an increase in reaction from 1 to 5 h for corn starch. Similarly for amaranth starch an increase in % succinyl from 1 to 5% with an increase in reaction from 1 to 5 h was observed (Fig. 3). The DS values paralleled the results obtained for % succinyl (Figs. 2 and 4, respectively, for corn and amaranth starch). A DS of 0.015 to 0.220 for CSS and 0.016 to 0.241 for the ASS was observed within the framework of parameters chosen in this study.

The increase in % succinyl and hence the DS with the reaction time could be due to the increased rate and time of collisions of succinic anhydride with starch through the formation of succinyl-pyridinum intermediate. Hence, attempts were made to carry out the succinylation by randomly increasing the reaction time of 1 h incremental time from 5 to 10 h under similar set of conditions. However no significant increase in the % succinyl and the DS was observed even after 10 h of reaction time (results not given). This could be due to maximum collisions taking place up to

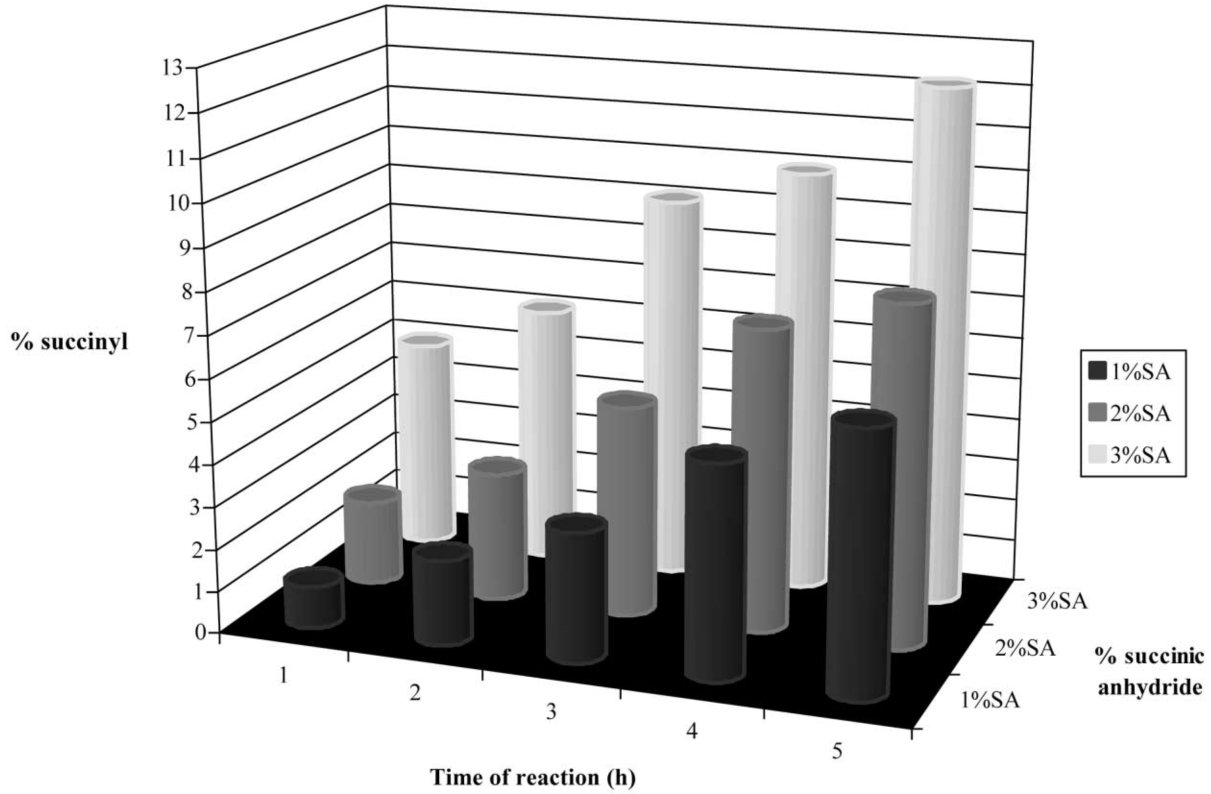

Fig. 1. Effect of reaction time on % succinyl of corn starch succinate at 1, 2 and 3% addition of succinic anhydride.

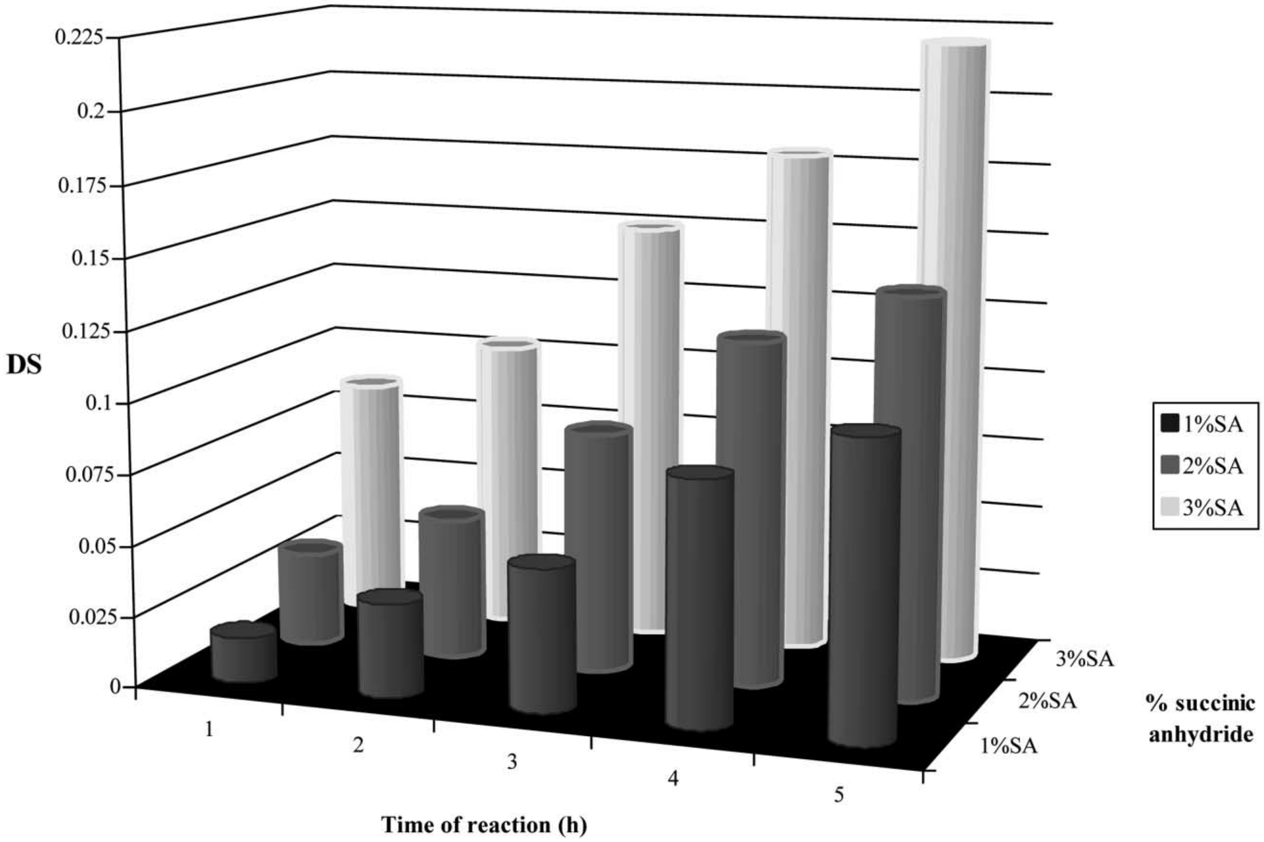

Fig. 2. Effect of reaction time on DS of corn starch succinate at 1, 2 and 3% addition of succinic anhydride.

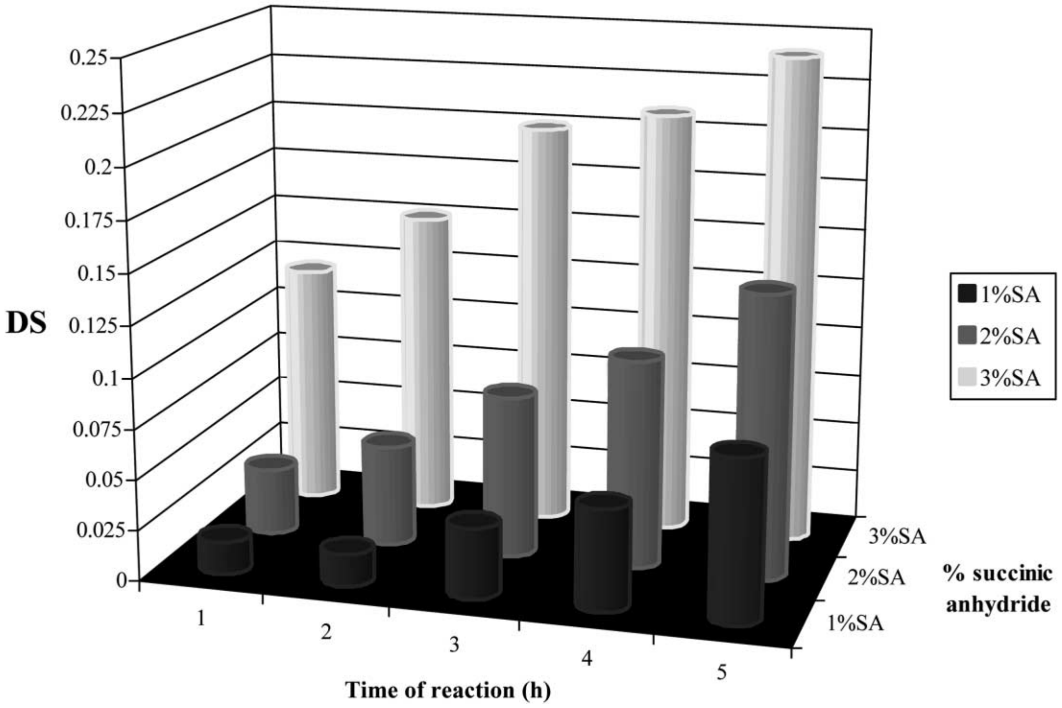

Fig. 3. Effect of reaction time on % succinyl of amaranth starch succinate at 1, 2 and 3% addition of succinic anhydride.

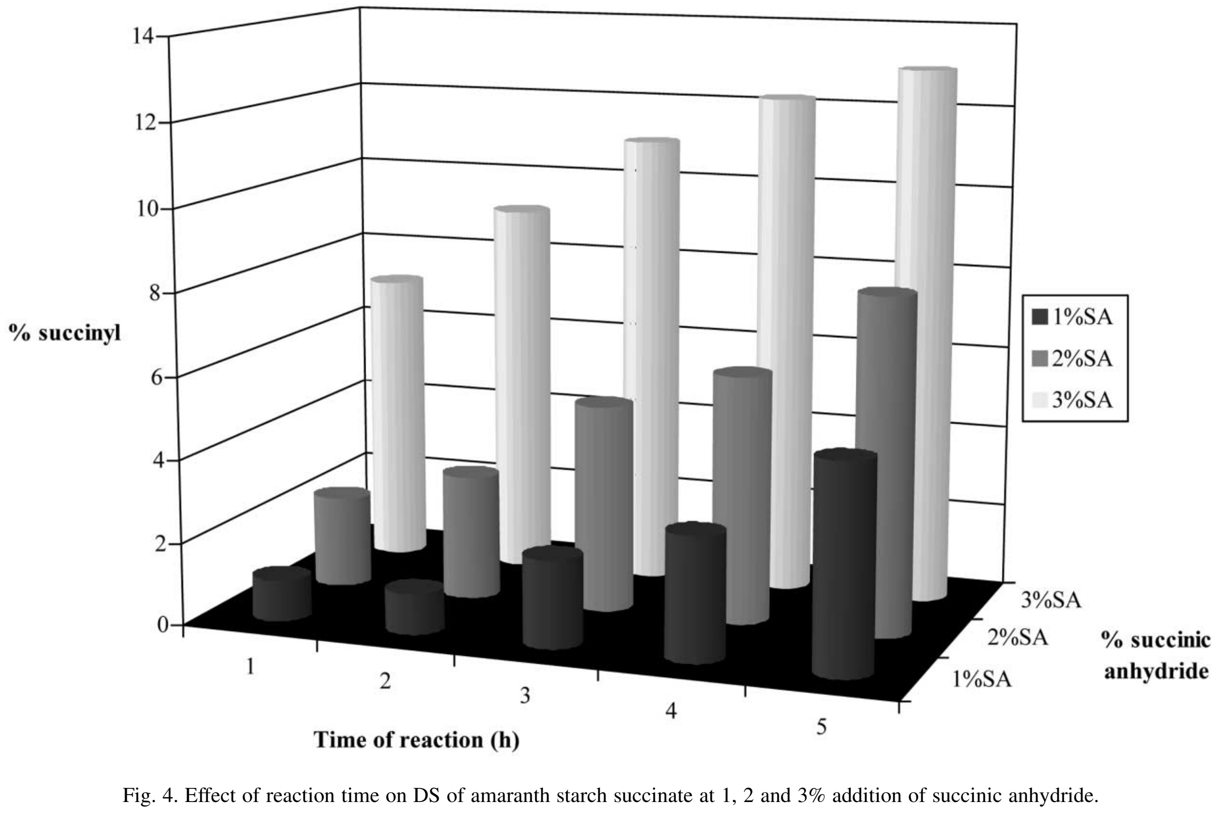

Fig. 4. Effect of reaction time on DS of amaranth starch succinate at 1, 2 and 3% addition of succinic anhydride.

Table 2
Conditions optimised for the preparation of CSS and ASS

| DS | CSS | | | ASS | | |
|---|---|---|---|---|---|---|
| | % Succinyl | Time of reaction (h) | % Succinic anhydride used | % Succinyl | Time of reaction (h) | % Succinic anhydride used |
| 0.05 | 3.05 | 2 | 2 | 3.02 | 2 | 2 |
| 0.10 | 6.13 | 5 | 1 | 6.00 | 4 | 2 |
| 0.15 | 9.10 | 3 | 3 | 9.12 | 2 | 3 |
| 0.20 | 12.21 | 5 | 3 | 11.01 | 3 | 3 |

Table 1
Effect of time of reaction on the DS and % succinyl of CSS and ASS at 3% addition of succinic anhydride using a starch/pyridine ratio of 1:1 (values are mean ± SD of three determinations)

| Time of reaction (h) | CSS | | ASS | |
|---|---|---|---|---|
| | % Succinyl | DS | % Succinyl | DS |
| 1 | 1.02 ± 0.026 | 0.016 ± 0.004 | 1.04 ± 0.041 | 0.016 ± 0.002 |
| 2 | 1.08 ± 0.056 | 0.016 ± 0.002 | 1.00 ± 0.101 | 0.016 ± 0.013 |
| 3 | 2.12 ± 0.026 | 0.033 ± 0.003 | 0.95 ± 0.451 | 0.015 ± 0.003 |
| 4 | 1.00 ± 0.11 | 0.016 ± 0.002 | 2.11 ± 0.050 | 0.033 ± 0.004 |
| 5 | 2.04 ± 0.06 | 0.033 ± 0.004 | 2.00 ± 0.072 | 0.033 ± 0.002 |

5 h of reaction time and stabilizing thereafter. Hence the reaction time of 5 h was considered as optimum for both the starches (Wolff et al., 1946; Mullen & Pascu, 1943).

Both CSS and ASS of low DS up to 0.20 could be obtained by the treatment of starch up to 3% succinic anhydride. The process so far optimised was based on the starch/pyridine ratio of 1:2. However, the high cost and toxic nature of pyridine are the deterrents for commercialisation. Therefore, pyridine recovery is a crucial economic factor. Mullen & Pascu (1943) have proposed a process for the recovery of pyridine. After the isolation of starch succinate by precipatition and washing with water, the aqueous pyridine azeotrope remaining is treated with lime. This can then be distilled to recover the pyridine. Alternatively the azeotrope can be broken by salting or solvent extraction with benzene. However none of these methods have been commercially used till date due to unfavourable economics of the system. An attempt was made in the present work to reduce the amount of pyridine used, by altering the starch/pyridine ratio from 1:2 to 1:1. The effect of this ratio on the % succinyl and the DS at varying times using 3% succinic anhydride for both corn and amaranth starches was studied. The results are shown in Table 1. It can be seen that the % succinyl and the DS fluctuated between 1–2% and 0.016–0.033, respectively, for corn starch. Similar fluctuation in results was observed for amaranth starch. Moreover both the starches showed the typical brownish black colour, indicating charring of starch due to insufficient pyridine available for the reaction mixture. Various problems were encountered in this step of optimisation. Incomplete succinylation was observed due to insufficient pyridine available for the reaction. The reaction mixture could not be stirred properly because of reduced quantity of pyridine leading to charring of starch. Therefore, a starch/pyridine ratio of 1:2 was found to be necessary for the reaction to run smoothly and was considered as optimum.

CSS and ASS of various DS were obtained in the above set of experiments. They were powdered and passed through a sieve of 60 mesh. The best conditions that could give CSS and ASS of DS 0.05, 0.10, 0.15 and 0.20 were evaluated and shown in Table 2. CSS and ASS so selected were stored in air tight containers until further use.

## 4. Conclusions

Starch succinates up to a DS of 0.2 could be prepared by varying succinic anhydride concentration and time of the reaction. Starch: pyridine ratio of 1:2 proved to be vital for the reaction to take place. There was no prominent effect of the starch type on the DS of the starch succinates. Such low DS starch succinates could find promising food and non-food applications and need to be studied.

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