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Synthesis and Characterization of low DS Succinate Derivatives of Cassava (*Manihot esculenta* Crantz) Starch

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Succinylation of cassava starch was carried out in aqueous medium to prepare derivatives with low degree of substitution (DS) and the physicochemical properties of the products were determined. A response surface design was used for the experiment with three levels of each of the three variables *viz.*, concentration of succinic anhydride, reaction time and pH of the reaction medium. The reaction was followed in terms of the DS of the products and reaction efficiency (RE). The degree of substitution of the derivatives varied from 0.001 to 0.022 and reaction efficiency from 2.2 to 46.8%. The DS and RE showed an adequate fit to a second order polynomial model of the variables used. Succinylation brought about increase in the swelling volume, peak viscosity and paste clarity of the starch. However, the pasting temperature and solubility did not show any significant change. The *in-vitro* α -amylase digestibility of the succinylated derivatives decreased in comparison to that of native starch and this decrease correlated with a corresponding increase in DS.

Keywords: Cassava starch; Succinylation; Response surface design; Physicochemical properties, Amylase digestibility

1 Introduction

The industrial use of native starches is limited because of their unstable pastes and gels. Therefore, starch is usually modified to make it easier to use, more stable during processing and to achieve a wider range of textures. Starches can be modified either physically or chemically [1]. Such modified starches generally have physicochemical properties that differ significantly from those of the parent starches, thereby increasing their usefulness in food and other industries [2]. Owing to its high viscosity and paste clarity, cassava starch is one of the commercially important starches. However, the paste and gel stabilities of cassava starch are low and hence it finds only limited application in the native form. Consequently, modified cassava starch is used widely in food and other industries. Succinylation by reacting starch with succinic anhydride is a commercially used chemical modification method [3].

Starch succinates offer a number of desirable properties such as high viscosity, better thickening power, low gelatinization temperature and good film forming properties.

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These succinates are used as binders and thickening agents in food, tablet disintegrants in pharmaceuticals and surface sizing agents in paper industry. The US Food and Drug Administration (FDA) permits reaction of starch with up to 4% succinic anhydride for food applications [4]. Since systematic studies on the succinylation of cassava starch are scanty, we have decided to examine the effects of succinic anhydride concentration, reaction time and pH of the reaction medium on the succinylation reaction of cassava starch in aqueous system and evaluating the physicochemical properties of the native starch and the modified starches. In this study, we used response surface methodology to analyze the effects of the variables on the succinylation of cassava starch and on the paste properties of the modified starches.

2 Materials and Methods

2.1 Experimental design

A Box-Benken design [6] for three factors was selected for the evaluation of the effect of pH, time and reagent concentration on the degree of succinylation and reaction efficiency. The design scheme consisted of 15 treatments whose central point was replicated three times for the calculation of the experimental error. A quadratic model

containing 10 coefficients was used to describe the responses, Y , observed to fit the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3, \quad (1)$$

where, b_i , b_{ii} and b_{ij} are the regression coefficients which show the nature and extent of the dependence of the responses on the associated reaction conditions and X_i the coded variables, linearly related to the reagent concentration, time and pH. The analyses were carried out using the statistical package SAS 8.01 [7].

2.2 Preparation of starch succinates

The various factors and their levels used were: concentration of succinic anhydride (3, 4 and 5%, based on starch dry weight), time (1, 2 and 3 h), and pH of the reaction medium (8–8.5, 9–9.5 and 10–10.5). Cassava starch was finely powdered, weighed out into a beaker and distilled water was added to achieve a concentration of 40% (w/v). The starch suspension was stirred using a mechanical stirrer in order to make it into a uniform slurry. The pH of the slurry was adjusted with a pH meter (Systronics, Bangalore, India) using calomel electrodes by adding dropwise a 3% solution of sodium hydroxide. To this slurry, a weighed quantity of succinic anhydride was added in small portions while simultaneously adding sodium hydroxide solution in order to maintain the pH of the medium at the desired level. Stirring was continued for the required reaction time. After the reaction was over, the pH of the medium containing the product was adjusted to 6.5 using hydrochloric acid (0.5 M). The product was recovered by vacuum filtration, washed with water and re-filtered. The washing was repeated three times and the product collected and dried overnight at 60°C in an air oven. The dried starch was ground using a mortar and pestle.

2.3 Analysis of the products

The level of succinylation of the modified starches was determined using the titrimetric method of *Whistler* and *Paschall* [8]. Degree of substitution (DS) and reaction efficiency (RE) were calculated according to *Wurzburg* [9].

$$DS = 162 \times \% \text{ Succinylation} / 10,000 - (99 \times \% \text{ Succinylation})$$

Where, 162 = molecular weight of glucose unit

10,000 = 100 × molecular weight of succinyl group

99 = molecular weight of succinyl group – 1

RE (%) = % Succinylation found × 100 / theoretical Succinylation

The pasting properties of the native and succinylated starches were determined using a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia). The slurries (10% by weight) were heated from 50 to 95°C at 12°C/min at 160 rpm, and were then held at 95°C for 2 min. Afterwards the pastes were cooled to 50°C at 12°C/min and finally kept at 50°C for 2 min. The peak viscosity, breakdown, final and setback viscosities, and pasting temperature were recorded.

The swelling volume and solubility of the starch pastes at 90°C were determined according to standard procedures [10, 11]. Briefly, 100 mg of starch was weighed into a 100 mL conical flask and 10 mL distilled water was added. The samples were kept in a boiling water bath for 20 min. After cooling, the samples were transferred to centrifuge tubes; the volume was made up to 10 mL and centrifuged at 2000 × g for 20 min. The height of the gel was measured and converted to volume of gel per unit dry weight of the sample [10]. From the supernatant 5 mL was transferred into a previously weighed Petri dish and dried in an oven at 60°C overnight. The weight of the residue was measured. The percentage solubility was determined [11] using the equation,

$$\text{Solubility (\%)} = \frac{\text{Weight of the residue (g)} \times (10 - \text{volume of swollen portion}) \times 100}{\text{Weight of the starch taken (g)} \times 5}$$

The paste clarity was determined by measuring the transmittance percentage (% T) of a 1% (w/v) solution of the starch at 700 nm after boiling in a water bath for 20 min [12]. The *in-vitro* α -amylase digestibility of the native and modified starches were determined using porcine pancreatic α -amylase with a digestibility unit of 500,000 (13.4 unit α -amylase / mg solids at pH 6–9, Sigma, St. Louis, USA) according to the method of *Singh* *et al.* [13], modified by *Padmaja* (personal communication). The sample (100 mg) was weighed; sodium phosphate buffer (10 mL, pH 6.9) was added and gelatinized on a boiling water bath. The samples were cooled; the enzyme solution (0.5 mL of a solution of 25 mg enzyme in 25 mL buffer) was added and incubated at 30°C for 30 min. After incubation, the samples were heated on a boiling water bath to deactivate the enzyme. The reducing sugar contents in the samples were determined as glucose by Nelson-Somogyi's method [14] and percentage digestibility was calculated.

2.4 Statistical analysis

The data were analyzed using the statistical package SAS 8.01 to perform ANOVA, to fit the second order polynomial equations and to generate surface plots using significant parameters ($p < 0.05$). Analysis of variance was done for analyzing a model which allows assessing the

relative values of different factors in modeling the variation in the responses. Coefficients of determination (R^2) were computed and the adequacy of the model was tested by separating the residual sum of square into pure error and lack of fit. Simple correlation coefficients were computed to analyze the effects of degree of substitution on the physicochemical properties of the modified starches.

3 Results and Discussion

3.1 Model analysis

The % succinylation, DS and RE were determined for all the 15 treatment combinations and are given in Tab. 1. The experimental values of % succinylation ranged from 0.08 to 1.36 and the DS values from 0.001 to 0.022. The reaction efficiency was between 2.2 and 46.8%. The highest values of the DS and RE (0.022 and 46.8% respectively) were obtained when starch was treated with 3% succinic anhydride at a pH of 9–9.5 for 1 h.

Tab 1. Percent succinylation and degree of substitution (DS) of succinylated cassava starches and reaction efficiency (RE).

Sample	Variables			Responses		
	Concentration [%]	Time [h]	pH	% Succinylation	DS	RE
1	3	1	9.0–9.5	1.36	0.022	46.8
2	3	3	9.0–9.5	0.83	0.014	28.6
3	5	1	9.0–9.5	0.92	0.015	19.3
4	5	3	9.0–9.5	1.16	0.019	24.3
5	3	2	8.0–8.5	0.91	0.015	31.4
6	3	2	10.0–10.5	0.34	0.006	11.5
7	5	2	8.0–8.5	0.91	0.015	19.1
8	5	2	10.0–10.5	0.21	0.004	4.4
9	4	1	8.0–8.5	0.91	0.015	23.6
10	4	1	10.0–10.5	0.08	0.001	2.2
11	4	3	8.0–8.5	0.42	0.007	10.8
12	4	3	10.0–10.5	0.19	0.003	4.9
13	4	2	9.0–9.5	0.67	0.011	17.5
14	4	2	9.0–9.5	0.58	0.010	15.2
15	4	2	9.0–9.5	0.63	0.010	16.3

The analysis of variance indicated that concentration of succinic anhydride, time and pH had significant ($p < 0.05$) influence on cassava starch modification. Nieto [15] reported similar results for the acetylation of *Xanthosoma violaceum* starch. Betancur et al. [16] observed significant

influence of reagent concentration, pH and their stronger interaction on the succinylation of jack bean starch.

A regression analysis was done of response variables as in Equation (1), which indicated that the experimental data had an adequate fit to a second order polynomial model ($p < 0.05$). The model illustrates the behavior of DS and RE as a function of succinic anhydride concentration (X_1), reaction time (X_2) and pH (X_3), as represented by Equations (2) and (3) and is depicted in Figs. 1 and 2. Only significant effects were retained in the fitted model as well as for drawing the response surfaces.

$$\begin{aligned} \text{Degree of Substitution} = & 0.0102 - 0.0013 X_2 \\ & - 0.00048 X_3 + 0.0052 X_1^2 + 0.0021 X_2^2 \\ & - 0.0056 X_3^2 + 0.003 X_1 X_2 + 0.0024 X_2 X_3 \end{aligned} \quad (2)$$

$$R^2 = 99.43$$

$$\begin{aligned} \text{Reaction Efficiency} = & 16.307 - 6.408 X_1 \\ & - 2.906 X_2 - 7.737 X_3 + 9.819 X_1^2 + 3.614 X_2^2 \\ & - 9.538 X_3^2 + 5.765 X_1 X_2 + 3.873 X_2 X_3 \end{aligned} \quad (3)$$

$$R^2 = 98.45$$

The reaction time and pH had negative main effects on DS, whereas all the three factors had negative main effects on RE. The quadratic terms of concentration and time and the interactions (concentration-time and time-pH) had positive effects, whereas pH² had negative effect on DS and RE. The determination coefficients (R^2) were high in both models which shows the adequacy of the mathematical models proposed to explain the reaction. These effects are visible in Figs. 1 and 2 which gives the effects of succinic anhydride concentration and reaction time at different levels of pH on DS and RE, respectively.

3.2 Physicochemical properties

Succinylation brought about a considerable increase in the swelling volume of cassava starch. The native starch had a swelling volume of 28.5 mL/g. The swelling volumes of the succinylated samples ranged from 29 to 45 mL/g (Tab. 2). There was a significant positive correlation of 0.59 between the swelling volumes and the degree of substitution of the modified samples ($p = 0.022$). In comparison to the unmodified cassava starch, the modified starches did not show a significant change in solubility. The solubility of the derivatives at 90°C ranged from 16.7 to 24.3%, whereas it was 21.6% for the native starch (Tab. 2). The solubility showed no significant correlation to the degree of substitution of the modified starches.

The gel clarity of cassava starch was improved by succinylation (Tab. 2). The % *T* was 21.4 for the native starch, whereas all the derivatives, excluding sample 10, showed

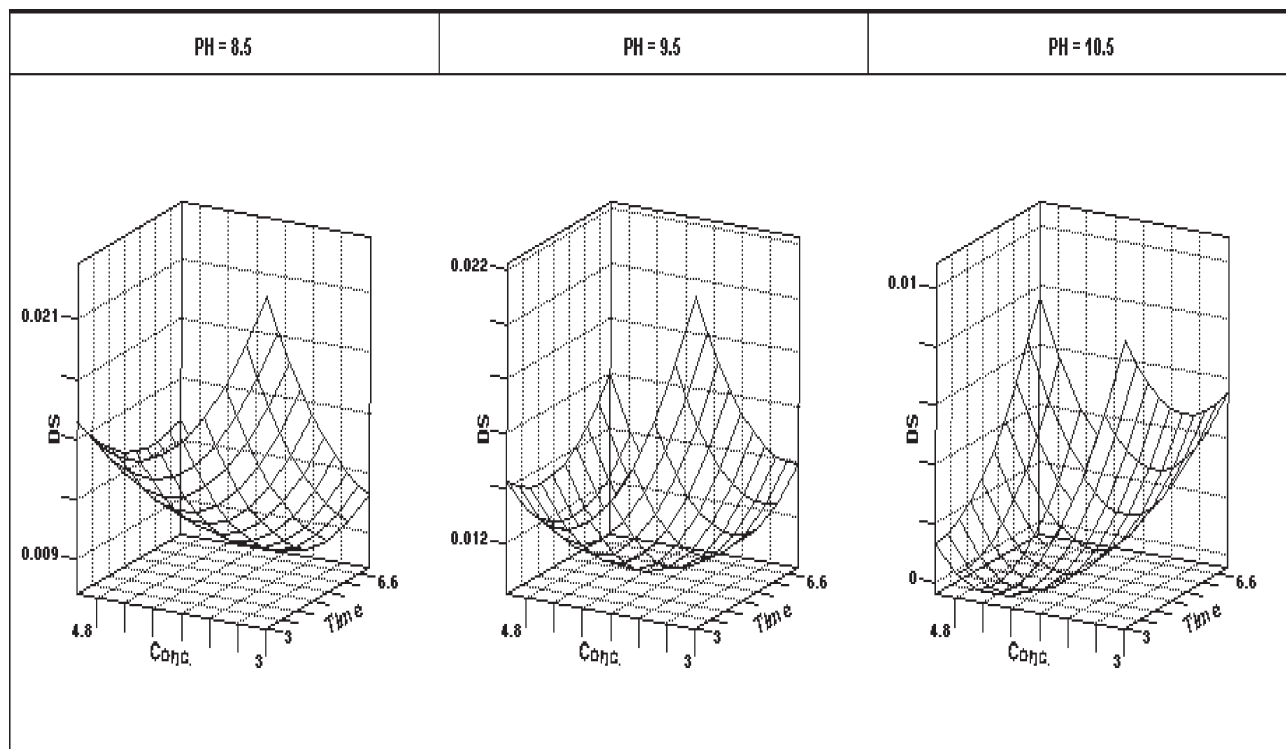


Fig 1. Response surface plots for the degree of substitution (DS) of succinylated cassava starches at different levels of pH.

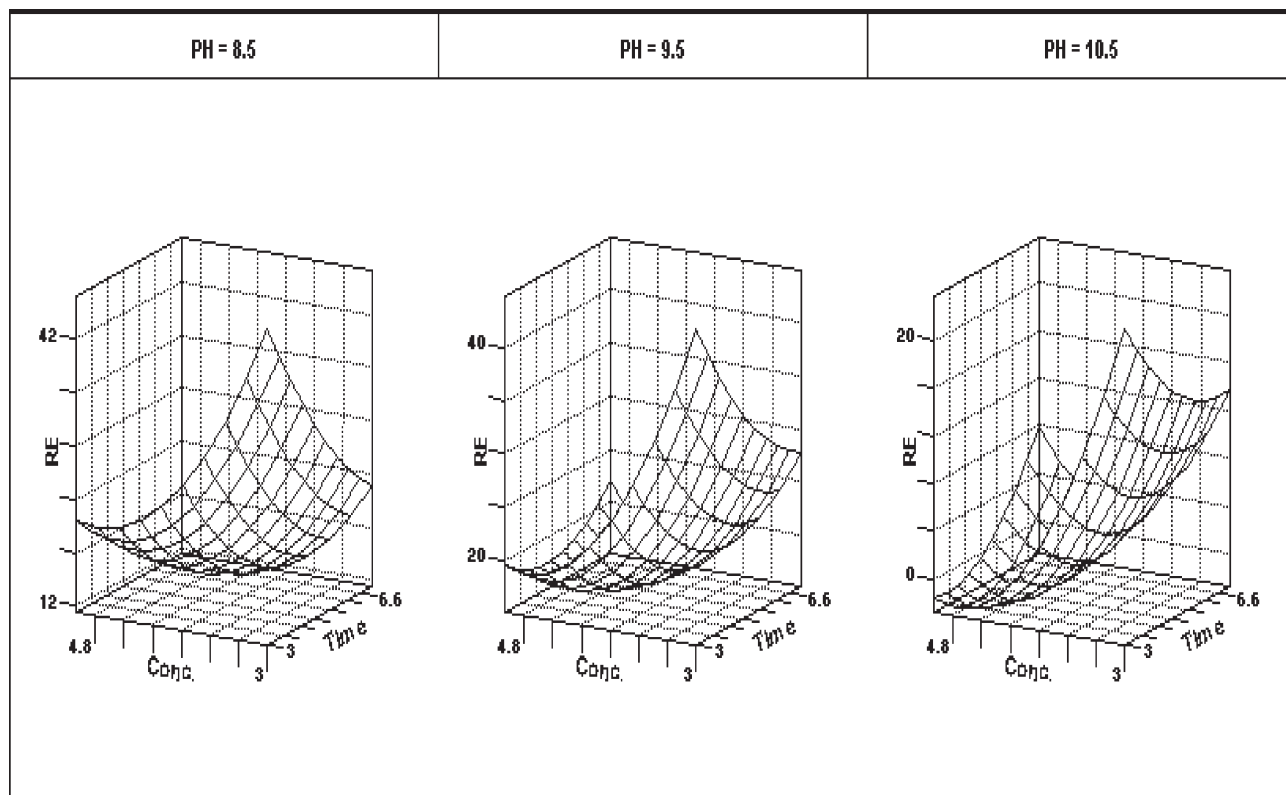


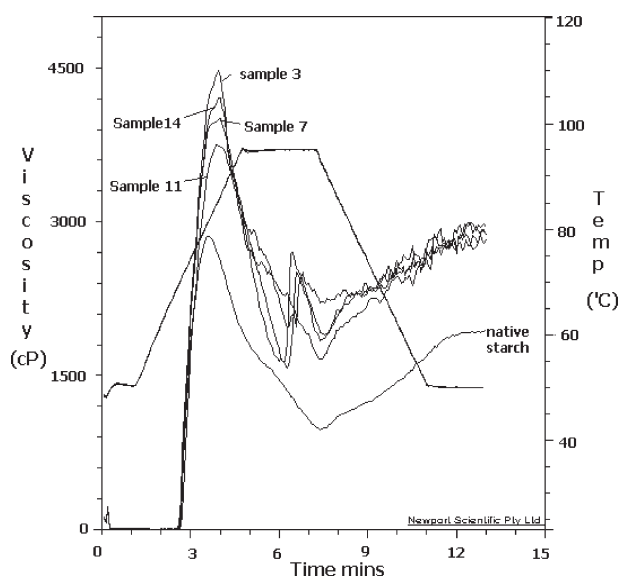
Fig. 2. Response surface plots for the succinylation reaction efficiency of cassava starch at different levels of pH.

Tab 2. Physicochemical properties of native and succinylated cassava starches.

Sample	Properties					
	Swelling volume [mL/g]	Solubility [%]	Paste clarity [%T]	Peak viscosity [mPa s]	Viscosity break-down ratio	Pasting temperature [°C]
1	44.00	24.24	28.9	4740	0.639	68.65
2	36.00	23.19	27.8	4175	0.647	68.85
3	45.00	19.94	26.4	4475	0.634	69.45
4	44.00	19.75	27.8	4534	0.574	67.85
5	44.00	24.30	26.5	4210	0.570	68.65
6	34.00	19.01	25.2	3688	0.557	69.40
7	45.00	16.97	27.6	4005	0.510	68.60
8	29.00	21.92	23.1	3202	0.649	68.75
9	42.00	16.74	26.8	3997	0.577	69.50
10	34.00	22.72	18.6	3234	0.675	68.35
11	44.00	24.33	25.0	3754	0.558	68.70
12	34.00	23.31	21.8	3275	0.657	68.75
13	45.00	20.91	25.9	4243	0.592	68.80
14	44.00	22.77	28.6	4208	0.627	68.80
15	45.00	19.11	28.3	3500	0.615	69.35
Native starch	28.50	21.63	21.4	2904	0.675	68.75

higher % *T* values (21.8–28.9%). The more diffused structure of the derivatives caused more light to pass through it, resulting in an increase in % *T*. *Bhandari* and *Singhal* [17] also reported increased paste clarity for the succinylated derivatives of corn and amaranth starches. The percentage light transmittance in the starch pastes of succinylated samples showed significant positive correlation ($p < 0.01$) to the degree of substitution ($r = 0.77$).

A significant increase was observed in the peak viscosity of the succinylated starch samples when compared to that of the native cassava starch ($p < 0.01$) (Tab. 2). The peak viscosity of the succinylated starches varied from 3202 to 4740 mPa s. The viscosity of the samples showed positive correlation to the DS ($r = 0.87$). The increase in viscosity might have occurred due to the decrease in the associative forces within the starch granules due to succinylation that resulted in an increased penetration of water during heating and ultimately an increase in viscosity. The RVA pasting curves of the native and succinylated starches (samples 3, 7, 11, 14) are given in Fig. 3. The samples with higher DS exhibited higher peak viscosity values. Starch succinylation normally reduces paste viscosity [18]. In contrast, in succinylated cassava starch, an increase in viscosity was observed with increase in % succinylation. Succinylation leads to the substitution of OH groups in the starch molecules with succinate groups. The repulsion between these bulky

**Fig. 3.** RVA pasting curves of native and succinylated cassava starches.

groups leads to the observed increase in viscosity and swelling volume. The bulky hydrophilic succinate groups will bring about starch chain expansion and viscosity increase. Similar results were obtained with cornstarch, where the effect was attributed to the presence of succinate groups [4]. *Betancur et al.* [16] reported an increase in viscosity of succinylated *Canavalia ensiformis* starch. *Sathe* and *Salunkhe* [19] also reported viscosity increase in *Phaseolus vulgaris* starch pastes when acetylated. The cassava starch, acetylated with acetic anhydride and 1% H_2SO_4 as catalyst is reported to exhibit enhanced viscosity of pastes [20]. This increase in viscosity imparts higher stability and clarity to the starch pastes.

Succinylation did not strongly influence the viscosity breakdown, since only a slight decrease was observed in the breakdown ratio, in comparison to that of the native starch. No significant correlation was observed between the breakdown ratio and DS of the derivatives. Compared to the unmodified starch, there was no significant change in the pasting temperatures of the succinylated samples (Tab. 2).

The *in-vitro* α -amylase digestibilities of the native and succinylated samples were determined and it was found that the succinylated derivatives possessed lower enzyme digestibility when compared to the unmodified starch. The % digestibility after 30 min of incubation of the starch with the enzyme at 30°C was 87.6% for the native starch, whereas it was only 63.9% for the succinylated sample having a DS of 0.015. Results proved that succinylation decreased the percentage of released reducing sugar resulting from α -amylase hydrolysis and this per-

centage decreased gradually as the degree of substitution increased in the succinylated samples. Similar results were obtained by Marcazzan et al. [21], who reported that α -amylase was active on the succinic esters of starch and amylose and that the activity showed a linear decrease with the number of succinic units introduced into the polysaccharide.

4 Conclusion

Cassava starch succinates were prepared in aqueous medium. Maximum values of degree of substitution (0.022) and reaction efficiency (46.8%) were obtained by succinylation of starch with 3% succinic anhydride at pH 9.0–9.5 for 1 h. In comparison to the native cassava starch, the modified starches obtained by this procedure exhibited enhanced swelling volume, viscosity, paste and gel clarity and lower *in-vitro* α -amylase digestibility. These properties make them good thickening agents and could also be used in foods like jellies, sausages and fruit pastes, which require transparency.

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