CHAPTER III

EXPERIMENTAL PROCEDURE

3.1 Materials

3.1.1 Tapioca Starch

Tapioca Starch from Siam Modified Starch Co., Ltd. was used.

3.1.2 Hydroxypropylating Agents

Commercial grade of propylene oxide from Siam Modified Starch Co., Ltd. was used as hydroxypropylating agent.

3.2 Instruments

The major instruments used are listed below.

1. Hewlett Packard General Spectrophotometry.
2. Viskograph Brabender 486044 Brabender OHG Duisburg.
3. Nuclear Magnetic Resonance (NMR) Spectra BRUKER ACF 200 MHz.
4. Kett Electric Laboratory C-100-3 Whiteness.
5. Sartorius Moisture Analyzer MA 30 from Scientific Promotion Co., Ltd.
6. JEOL JSM 5800LV Scanning Electron Microscope.

3.3 Experimental Procedure

3.3.1 Preliminary Study of Hydroxypropylation of Tapioca Starch

The native tapioca starch was added into water at 40°C with vigorous agitation (40% dry starch weight in water). After that, aqueous sodium hydroxide 3.5% w/v was added dropwise. Then propylene oxide was slowly added. The reaction mixture was stirred at 40°C for 24 hrs. After that, the reaction mixture was neutralized with 1:1 ratio of HCl : H_2O and filtered. The modified starch was thoroughly washed with water and dried at 50 °C for 6 hrs.

3.3.2 Effect of Sodium Sulfate on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.1. But 8, 12 and 15% by dry starch weight of sodium sulfate was added into the mixture. Degree of substitution of hydroxypropylated starch was measured by colourimetry.

3.3.3 Effect of Sodium Hydroxide on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.2 using the quantity of sodium sulfate, giving the highest degree of substitution. However, the quantity of sodium hydroxide was changed into 0.5, 1.5 and 2.0% dry starch weight.

3.3.4 Effect of Propylene Oxide Concentration on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.3 using the quantity of sodium hydroxide, which gave the highest degree of substitution, was used. The propylene oxide concentration was altered to 5, 9 and 20% dry starch weight.

3.3.5 Effect of the Reaction Time on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.4 using the propylene oxide concentration, giving the highest degree of substitution. However, the reaction time was changed into 6, 12, 18 and 20 hrs.

3.3.6 Effect of Temperature on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.5 using the reaction time, which gave the optimum degree of substitution, was used. The temperature was altered to 30, 45 and 50°C.

3.3.7 Effect of the Reaction Medium on Degree of Substitution

Hydroxypropylated tapioca starches were prepared according to 3.3.6 using the temperature, which gave the highest degree of substitution, was used. However, the medium in this reaction was changed into aqueous ethanol 20, 30 and 70 % by dry starch weight but sodium sulfate was not added in the reaction.

3.3.8 High Degree of Substitution of Hydroxypropylated tapioca starch

The native tapioca starch was added at 45°C in 70% ethanol medium with vigorous agitation (starch concentration 40% by dry starch weight). After that, 3.5%w/v sodium hydroxide (2% by dry starch weight) was added dropwise. Then 15% propylene oxide was slowly added. The reaction mixture was stirred at 45°C for 24 hrs. The starch slurry was neutralized with 1:1 ratio of HCl : H_2O and filtered. The modified starch was thoroughly washed with water and dried.

3.4 Determination of Degree of Substitution by Colourimetry

3.4.1 Preparation of Standard Curve

A calibration curve with 1 ml aliquots of propylene glycol containing 25, 50 and 75 μg of propylene glycol/ml was also prepared. These standards were determined according to 3.4.3.

3.4.2 Sample Preparation

Known amount of hydroxypropylated starch sample (0.1 g) were weighed into 100 ml volumetric flask. 25 ml of 2 M sulfuric acid was added. The flask was then placed in a boiling water bath and was heated until the sample dissolved. It was then cooled to ambient temperature and diluted to 100 ml with water. These solutions were analyzed according to 3.4.3.

3.4.3 Analysis

1 ml aliquots of the solutions were transferred into 25 ml volumetric flask. Concentrated sulfuric acid (8 ml) was added dropwise, immersing the volumetric flask in cold water and mixed well. Flasks were then placed in a boiling water bath for 3 minutes and transferred immediately to ice bath. 0.6 ml of ninhydrin reagent was then added to the chilled solution and it was shaken well. Then they were placed in a 25 °C water bath for 90 minutes and volume was made to 25 ml with concentrated sulfuric acid. Absorbance for each flask was then measured at 590 nm using the unmodified starch as the reference.

3.5 Determination of Degree of Substitution by ^{1}H-NMR

Dry hydroxypropylated tapioca starch 0.01 g was dissolved in 2 ml D_2O. Vigorous shaking with heat resulted in a clear solution, which was transferred to a NMR tube. Spectra were obtained from 200 MHz Bruker ACF Spectrometer.

3.6 Examination of Physical Properties

3.6.1 Gelatinization Temperature

The Viskograph Brabender Model 486044 was used to determine the pasting properties of starch samples. Hydroxypropylated tapioca starch 6% by dry starch weight and 500 g of distilled water were combined and stirred in the aluminium sample canister. A programmed heating and cooling cycle was used, where the sample was heated to 50°C and heated again to 95 °C in 30 minutes, held at 95 °C for 30 minutes, cooled to 50 °C in 30 minutes and then held at 50 °C for 10 minutes. Finally, the viscograph was performed.

3.6.2 Moisture

The Sartorius Moisture Analyzer Model MA 30 was used to determine the moisture of starch samples. 5 g of hydroxypropylated tapioca starch was heated in the moisture analyzer. Moisture of starch was expressed as percent moisture in starch.

3.6.3 Starch Morphology

The SEM at 15 kV accelerating voltage, and detected the electron with backscattered electron emission detector examined the morphology of the hydroxypropylated tapioca starch.

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