CHAPTER II

EXPERIMENTAL

1 Materials

All of the materials employed in this study were obtained from commercial sources and used as received:

> Glutinous Rice Flour (Cho-heng, Thailand)
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> Rice Flour (Cho-heng, Thailand)
>
> Tapioca Starch (Chao Phya, Thailand)
>
> Corn Starch (Thailand)
>
> Mung Bean Flour (Thailand)
>
> Sodium Carbonate, Anhydrous (BDH, England)
>
> Sodium Hydroxide (Eka Nobel, Sweden)
>
> Hydrochloric Acid (E. Merck., Germany)
>
> Starch 1500^R (Colorcon., USA)
>
> Eratab^R (Choheng, Thailand)

2 Methods

2.1 PRELIMINARY INVESTIGATION ON MODIFICATIONS OF VARIOUS STARCHES

Physico-chemical modification of various starches were carried out by methods as follows.

2.1.1 Crosslinking of Starches

Each variety of the five locally available starches, rice, glutinous rice, mung bean flour, corn, and tapioca starch was modified by crosslinking reaction for 6 hrs.

The following composition of materials used in crosslinking process was used.

Dry Starch or Flour	350.0	g
Sodium Trimetaphosphate	11.0	g
Sodium Carbonate Anhydrous	10.0	g
Sodium Hydroxide	3.7	g
Purified Water	450.0	g

Dry starch or flour [about 10-12 % moisture content] was dispersed in water in which sodium trimetaphosphate and sodium carbonate anhydrous had already been dissolved to and adjust the pH of the slurry to 11.0 or more with sodium hydroxide. The slurry was heated to 50 $^{\circ}$C with continuous stirring and kept at this reaction temperature for 6 hrs. After the reaction completed, the slurry was neutralized with hydrochloric acid (10 N), washed with plenty of water, filtered and dried.

2.1.2 Preparation of Starch Aggregates by Spray-drying Techniques

Finally, the modified starches attained from 2.1.1 was spray-dried under the conditions described below, using laboratory-type spray-dryer apparatus (Niro Atomizer, Denmark).

Starch dispersion was prepared by dispersing dry starch obtained from 2.1.1 (about 12 % moisture content)in water with the aid of homogenizer(Silverson, England) until homogeneous dispersion was obtained. The slurry was subsequently spray-dried under various conditions as follows.

- Concentration of starch dispersion: 50 % w/w

- Drying inlet air temperature : 130 $^{\circ}$C

- Feed rate : 12.96 g/min

- Atomizing pressure : 1.5 bar

Compressibility, disintegration time and angle of repose of the starch aggregates obtained were evaluated according to the methods which would be described later. The starch which yielded the best results was selected for further studies.

2.2 INVESTIGATION ON RICE FLOUR MODIFICATIONS

After completion of the preliminary investigation,

it was found that rice starch showed the most satisfac
tory compressibility, disintegration, and flowability
among the starches modified. So only rice starch was
selected for furthur investigation to improve its
properties.

Rice flour was modified by various methods as
follows.

2.2.1 Preparation of Spherical Starch Aggregates by Spray-drying Techniques

Rice flour was spray-dried under various
conditions as follows.

Procedures

Starch dispersion was prepared by dispersing
rice flour (about 12 % moisture content) in water with
the aid of homogenizer (Silverson, England) until
homogeneous dispersion was obtained. The slurry was
subsequently spray-dried(Niro Atomizer, Denmark) under
various conditions as follows.

- Concentration of starch dispersion: 30, 40,
50 % w/w

- Drying Inlet Air Temperature : 130, 135 $^{\circ}$C

- Feed Rate : 4.32, 12.96, 21.6 g/min

- Atomizing Pressure : 1.0, 1.5, 2.0 bar

In order to study the effects of each

variable in spray-drying process, it was necessary to keep the other variables constant, and varied only the desired variable. To study the effects of concentration on physical and tabletting properties, the concentration was varied while the temperature, feed rate, and atomizing pressure were fixed. In the case of the effects of temperature, feed rate, and atomizing pressure, the experiments were carried out in the same way.

2.2.2 Effects of Concentration of Starch Slurry

The concentrations used were varied from 30, 40, up to 50 % while the temperature, feed rate, and pressure were kept at 130 C, 12.96 g/min, 1.5 bar respectively. Subsequently, physical and tabletting properties of spray-dried starch aggregates obtained were evaluated and the concentration that gave the best results i.e., highest compressibility, shortest disintegration time and lowest angle of repose (highest flowability) was selected to be the optimum concentration to be used later.

2.2.3 Effects of Inlet Air Temperature

Temperatures used were 130 and 135 $^{\circ}$C while

feed rate was 12.96 g/min, while maintained atomizing pressure at 1.5 bar with optimum concentration value from the study in 2.2.2. The spray-dried product was evaluated in the same way as 2.1.5 and the optimum temperature was selected based on the same criteria.

2.2.4 Effects of Feed Rate

Experimental feed rates used were 4.32, 12.96, and 21.6 g/min. Pressure was set at 1.5 bar, the concentration and temperature were the same as the study in 2.2.3. The products were evaluated and the optimum feed rate was selected in the same way as described earlier.

2.4.5 Effects of Atomizing Pressure

Atomizing pressures at 1.0, 1.5, 2.0 bar were used, along with the optimum concentration, optimum temperature and optimum feed rate obtained earlier. The optimum atomizing pressure was selected as previously described.

2.3 Crosslinking of Rice Flour

Rice flour was modified by crosslinking reaction for various time intervals. The composition of materials used in crosslinking process was as

described in 2.1.1. The crosslinking reaction time ranged from 2, 6, 10 hrs.

The crosslinked rice flour was subsequently evaluated to determine the degree of crosslinking as described later in 2.7and spray-dried under the optimum conditions attained from the study in 2.2.1 and the starch aggregates obtained were evaluated as described in 2.5 and 2.6 below.

2.4 Deproteinization and Crosslinking of Rice Flour

In order to improve the tabletting properties of rice starch aggregates, another fraction of rice flour was modified by deproteinization and crosslinking (as 2.2) respectively. Finally, the modified rice flour was spray-dried and evaluated as before.

Procedures of Deproteinization

300.0 g dry starch (rice flour) was dispersed in 500 ml water which had been dissolved 2 g sodium hydroxide and mixed for 5 min. in a blender (National, Japan). The slurry was transferred and heated to 50 ^{o}C and held for 3 hrs. with agitation at this temp. Then it was centrifuged at 2,000 RPM by supercentrifuge (Hitachi,Japan) for 15 min. The starch

sediment was collected and neutralized with hydrochloric acid, washed, filtered and dried.

2.5 Evaluation of Physical Properties of Starch Aggregates

2.5.1 Size and Shape

Samples of starch aggregates were coated with gold prior to the microscopic examination using ion sputtering and then photographed at appropriate magnification by scanning electron microscope(Jeol,JSM-35 CF, Japan).

2.5.2 Particle Size Distribution

Particle size distribution was determined by sieve analysis. Ten grams of starch aggregates were put on the top of sieve series ranging from 425, 250, 180, 150, 125, 106, 90, 75, 45 μm respectively. The nest of sieves (Endecotts Ltd, England) was placed on the sieve shaker (Josef Deckelman, Germany) for 10 minutes. The results, which averaged from two determinations, were reported as percentage of weight retained on each sieve size.

2.5.3 Bulk, Tapped Density and Percent Compressibility Determination

The bulk and tapped density were determined from the accurate weight of about 30 g. of starch aggregates carefully charged into a 100 ml graduated cylinder and bulk volume was recorded. Division of weight by bulk volume showed bulk density. Tapped density was performed by dropping graduated cylinder on a hard wood surface from a height of about 5 cm. until a constant volume was obtained. Division of weight by this volume showed tapped density. Both densities were averaged from 3 determinations. The compressibility was calculated from the following equation.

% Compressibility = $\dfrac{\text{(Tapped Density-Bulk Density)}}{\text{Tapped Density}}$ x 100

2.5.4 Moisture Determination

The moisture content of starch aggregates was determined by using Ohaus moisture determination balance. About 3 g. of starch aggregates was spread uniformly in a thin layer and accurately weighed on a pan, then it was exposed to an infrared lamp at 1.5 "

from the pan and intensity of 20 Watt until constant weight was obtained. The percent moisture content was calculated based on the following equation.

$$\% \text{ Moisture Content} = \frac{(\text{Wet Wt. } - \text{ Dry Wt.})}{\text{Wet Wt.}} \times 100$$

2.5.5 Angle of Repose Determination

Angle of repose was determined by cylinder method. Appropriate amount of starch aggregates was carefully filled in the cylinder, which was placed on the graph paper, until it was filled at the top of cylinder, then slowly lifted the cylinder in the vertical way, producing round heap. Angle of repose was calculated from the following equation.

$$\text{Angle of Repose} = \tan^{-1} H/R$$

H and R are the height and radius of heap respectively.

2.5.6 Flow Rate Determination

Accurate weight of about 30 g. of starch aggregates was filled in a glass funnel with 8 mm.

internal stem diameter fixed on a clamp. The time was recorded when the starch aggregates started to flow until finished. The flow rate was expressed in g/ second.

2.6 Evaluation of Tabletting Properties of Starch Aggregates

2.6.1 Preparation of tablets

Starch aggregates were compressed into tablets by Carver Laboratory Press. 300 mg of starch aggregates without any adjuncts was accurately weighed and compressed under forces ranged from 1,000; 1,500; and 2,000 lbs. using a 10.1 mm. in diameter, round, flat faced punch. The compressional forces were maintained for 1 second and then quickly released.

2.6.2 Weight Variation

Twenty tablets were individually weighed and average weight was calculated.

2.6.3 Compressibility (Hardness)

The hardness was measured using the Schleuniger -2 E hardness tester and the mean of ten tablets was calculated.

2.6.4 Thickness

The thickness was measured by using micrometer. The mean was averaged from 10 tablets.

2.6.5 Friability

Twenty tablets or not less than 6 g. were weighed accurately and transferred into the Roche friabilator rotating at 25 RPM for 4 min. then they were dedusted and reweighed. Friability was calculated as percent weight loss.

2.6.6 Disintegration Time

Disintegration time was determined according to USP specifications using Hanson disintegration apparatus. The average was calculated from 6 tablets.

2.7 Degree of Crosslinking

The degree of crosslinking was measured indirectly by determining the swelling power and viscosity of crosslinked starch. The more the degree of crosslinking, the less the swelling power and viscosity (Rutenberg,1980).

2.7.1 Swelling Power

Accurate weight of about 1.0 g. of dry starch was employed and 6 ml. of water was poured and mixed well in a test tube. It was then immersed in a water bath and held at 80 C for 5 min. The starch gel was centrifuged at 1,000 R.P.M. for 10 min. The supernatant was poured off and the weight of swollen sediment determined. Swelling power was determined from the following equation.

$$\text{\% Swelling Power} = \frac{\text{Wt. of Swollen Sediment} \times 100}{\text{Wt. of Dry Starch}}$$

2.7.2 Viscosity

Twenty grams of dry starch was dispersed in 100 ml water and the slurry was transferred to Brabender viscoamylograph. The measurements of viscosity were performed at the three cycles respectively as follows.

1. Heating cycle: heat the starch dispersion from room temperature(25 $^\circ$C) to 95 $^\circ$C.

2. Holding cycle: hold the temperature at 95 $^\circ$C for 30 minutes.

3. Cooling cycle: let the dispersion cool down from 95 $^\circ$C to 40 $^\circ$C.

The heating rate was 3 $^\circ$C/min. The stirring rate

was 75 rpm.

2.8 Comparative Commercial Modified Starch Products

Starch 1500^R and Eratab^R were used as comparative materials in evaluation of the properties of starch products (as described in 2.2-2.7) prepared by the experiments under this investigation.