The Study on the Hydrocolloid Extraction Methods From Samrong(Sterculia lychnophora Hance)

By

Mr. Chang Ben Yeh

A special project submitted to the Faculty of Biotechnology, Assumption University in part fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology

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ABSTRACT

Samrong, *Sterculia lychnophora,* was a commonly found in Thailand. Its amazing water holding capacity leads researchers to believe that the hydrocolloid was presented in the seed. However, the report on this species is limited. In this project, the objectives were to study a possible method to extract crude hydrocolloid from Samrong seed and the study of extracted powder viscosity quality.

The research was started to ground seed as a powder, then boiled in water for three hours. The swollen mucilage was collected and soaked with 0.5 M NaOH solution for one hour. The crude gum was precipitated by ethyl alcohol, then dried in an oven and ground into powder of crude Samrong gum.

In order to evaluate the quality of crude gum, it was tested by many parameters for viscosity adjusted as a guideline. Dried powder was dispersed in various conditions (pH, salt, temperature and mixing method) for determination of flow rate as viscosity characteristics.

The result was shown that the percent yield of extracted powder could be increased from 1.99 % (w/w) to 38 ~ 40 % (w/w) of Samrong powder by the final extraction method. It was required the heating step in the extraction method to gain the hydrocolloid powder that would thicken in distilled water. The volumetric flow rate also achieved to 0.05 ml /sec.

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INTRODUCTION

Samrong or Pandahai seed (*Sterculia lychnophora* Hance) is a common edible plant which found mostly in the Eastern of Thailand. It is a traditional herb in the Southeast Asia and China. The dried Samrong seeds are soaked with hot water for a few hours until completely swellings. They act as medicine for many sicknesses such as asthma, fever, throat swelling, and constipation. (Kanit, 2004)

There are many Samrong beverages as a healthful beverage in Thailand. They usually mix with other herbs to enhance the functions like many herb beverages.

This project was studied the possibility extraction method of crude hydrocolloid from Samrong. Recently, there hasn't been found any research reporting the hydrocolloid extraction from Samrong like other gums. If this project success, The crude hydrocolloid may be develop to be the new hydrocolloid like thickening agent, adhesive (glue), and gelling agent in many industries.

OBJECTIVES

1. To study a possible method to extract crude hydrocolloid from Samrong by physio-chemical method.

2. To study the viscosity of the dried powder under various conditions.

LITERATURE REVIEW

Name (Salisa, 2004)

Scientific name

- *Sterculia lychnophora*
- *Scaphium macropodum beaum*
- *Semen scaphii lychnophoria*
- *Sterculia scapigera wall*

Common name

- Samrong (Thai)
- Pangdahai (Chinese)
- Boat-Fruited Sterculia seed (English)

Characters

Figure 1 : The Semen Sterculiae Lychnophorae leave

Source: http://www.fzrm.com/plantextracts/Boat-fruited_Sterculia_Seed_extract.htm.

Sterculia lychnophora Hance tree (Figure 1) is tall about 30-40 m. Leaves shape like coriaceous, glossy, glabrous, entire, base rounded or nearly

truncate and often cordate. It's about 6-20 cm. long and 4-10 cm. wide. The inflorescence, terminal panicle and calyx are deeply lobate. It has very short tube but the stamina column is longer than the calyx tube. Generally, stamina variable has more numerous in the male flowers. In the hermaphrodite flowers, they are the ovary villous, and 2-5 carpals, membranous, monospermous, stigma sessile or nearly bilobate. It has 1-5 follicles, 15-20 cm long and 4-5 cm. wide. The seeds are officinal and have 2 cm length ovoid, glabrous, and shiny (Figure 2). The taste is sweet. The seeds contain mucilage and traces of theobromine and caffeine. (Keys, 1996)

Figure 2 : The Sterculia lychnophora Hance fruit

Source: http://www.fzrm.com/plantextracts/Boat-fruited_Sterculia_Seed_extract.htm.

Figure 3 : Boat-Fruited Sterculia seed without drying

Source: http://www.maima.com/kain/images/fy04.jpg

Figure 4 Boat-fruited Sterculia Seed

Source: http://www.fzrm.com/plantextracts/Boat-fruited_Sterculia_Seed_extract.htm.

Samrong seed consists of four components layers - skin, mucilage layer, coated shell and endosperm.

Whole Seed Shell – The color of fresh fruit is pale green. After drying, the seed is turned to dark brown and shrunk. Shell and the mucilage layer are closely attached. The dried seed absorbed water very well after immerse it into water for a few minutes. The shell is separated from mucilage layer easily after swelling.

Mucilage layer – Mucilage has light brown layer with black dot and fragile in the dried seed. It forms spongy structure like jelly with absorbed water inside after rehydration. This section might contains hydrocolloid (non-starch hydrocolloid). (Figure 4)

Seed shell – It is a thin brown color layer and attached with inner seed endosperm. It doesn't absorb water.

Seed endosperm – It is yellow brown color. After immersed into water for some period, it can not absorb water.

Figure 5 : A swelling Sterculia lychnophora Hance seed

Source: http://waynesword.palomar.edu/images/stergm1b.jpg (picture)

Properties of Samrong seed

Poocharoen and Singpracha, 2003, reported the dried fruit powder could be rehydrated after the fruit was dried from swollen in the oven. The water holding capacity was 92.13 times of dry powder weight of the seed (Figure 4). Dry powder of the fruit contained 7.67 % w/w of soluble dietary fiber and 89.88 % w/w of insoluble dietary fiber out of total 97.55 %, others is ash.

Samrong is the mature seed from Sterculia plant; it is an important medicine that treats asthma, fever, throat swelling, and constipation. Chen J, et. al reported that polysaccharide pp III was isolated and purified from aqueous extract of the seeds of *Sterculia lychnophora*. It was composed of galactose, arainose and rhamnose, the molar ratio being 1: 1.67: 1.01 and molecular weight 162200. Rhamnoses in the main chain are linked by alpha-(1→3) glycoside linkage. PP III C is its major fragment, and is composed of galactose and rhamnose with a molar ratio of 1: 2.78 and MW 62500.

Two alkaloids, named sterculinine I and sterculinine II, together with thirteen known compounds were isolated from the ethanol extract of a well-known Chinese traditional drug, Pangdahai (the seeds of *Sterculia lychnophora* Hance). Their structures were elucidated by NMR, UV, IR and MS spectroscopic analysis.(Wang,2002)

Two alkaloids, named sterculinine I and sterculinine II, together with thirteen known compounds were isolated from the ethanol extract of the seeds of *Sterculia lychnophora.*

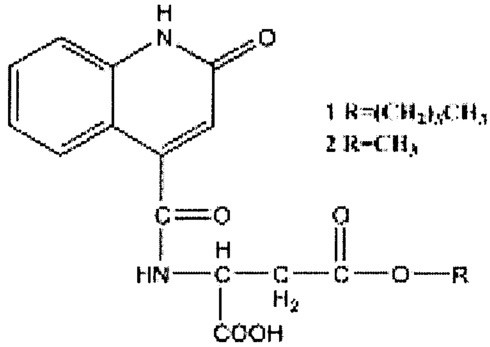


Figure 6: Structure of sterculinine I and sterculinine II.

Application

Medical Applications (Kanit, 2004)

Action

- To remove heat from the lung,
- To cure sore throat,
- To counteract toxicity, and
- To relax the bowels.

Indications

- Hoarseness of voice, dry cough, and sore, dry throat due to heat in the lung; constipation with headache and blood-shot eyes.

Usage

To be soaked in boiling water or decocted.

- It is used to treat dysentery, intestinal infection, coughing and sore throats. The seed is considered to be an economical emollient. An infusion is employed very satisfactorily to treat light bronchitis.

- The part of mucilage obtained from soaking the seeds in the water for a few hours or overnight is the remedial for many sicknesses such as phthisis, hemorrhage from the nose, stomachache, bowels or bladder, to counteract poison, sunstroke, ophthalmia toothache, intestinal worms, hemorrhoids, dry cough and fever in the marrow.

Food Applications (Kanit, 2004)

- When a seed of *Sterculia lychnophora* is soaked in water for several days, it imbibes water and swells to more than eight times its original volume. The seed coat expands into an edible gelatinous mass (carbohydrate gum) that is used for a beverage in Cambodia. According to S. Facciola (Cornucopia II, 1998), the cooling beverage is called "Sam-rong," and the flavor is enhanced with sugar and a flavoring such as jasmine or banana water.

- The Samrong is locally used as a laxative. It is develop to an instant fiber-enriched dried beverage. These advantages used specific for the patient who don't want carbohydrate or person who had diabetes. (Poocharoen and Singpracha, 2003). It also reported to uses as imitation bird-nest beverage which is very interesting one.

Gum Karaya (*Sterculia urens*) (Cornucopia II.1998)

General introduction of exudates gums

The earliest gums used were exudates gums because they were nearest to hand. They could be picked form trees or shrubs and easily dried and transported. Those that arose to prominence in ancient times and are still of industrial importance are Arabic, Ghatti, Karaya, and Tragacanth.

Exudates gums require much labor for incising, or tapping, the plant; for picking the dried or semidried gum; for sorting, bagging, and shipping; and, after reaching the gum importers, for grinding, sifting, or air classifying to remove particulate impurities and in some cases for further purification and spry-drying.

Production of an exudates gum is a natural defense mechanism of plants that seals bark wounds. On injury to the bark, many plants, particularly those that grow in semiarid regions, exude an aqueous solution thickened with gum that tends to cover the injury and harden to prevent infection and desiccation of the plant. Tragacanth gum is so quickly exuded and undergoes hardening so rapidly that its protection is not as effective as the gums of other plants. The initially sticky and soft gum exudates can trap insects, dust, and other wind blown debris. Where the gum touches the bark, it may absorb tannins and develop a degree of yellow color. Thus, commercial exudates gums require sorting and mechanical removal of impurities. Excessive impurities and color in gums cause them to receive a low grade classification.

Because of the extensive need of hand labor, the low gum yield per plant, and the nonagricultural production of most producing plants, it is inevitable that exudates gums of the ancient world, and now of the present time, will decrease in quantity used.

Introduction

Gum karaya is exuded form *Sterculia urens*, a large bushy tree growing to about 10m in height on the dry rocky hills and plateaus of Central and Northern India. Originally introduced as a substitute for gum Tragacanth, many uses were found for it and its commercial quantities rapidly increased until its use became second only to that of gum Arabic. A large increase in gum harvest is unlikely because no agriculturally produced trees exist and because of the long tie period needed to grow trees for substantial gum production. Some Sterculia grows in Africa, providing a small source that could possibly be increased.

Source

As with other exudates gums, production is increased when trees are incised or blazed. Commonly, young trees are tapped; but in larger trees, several incisions may be made. Gum begins to flow immediately; most of it exudes in the first 24 hours in the form of large irregular droplet-shaped masses that may weigh around 1 kg or more. The best quality of gum is obtained in April, May, and June as the temperature increases. Collection may be repeated after the monsoons in September, although this gum may be darker in color and lower in viscosity.

Structure

Natural gum karaya is partially acetylated with about 8% acetyl groups. It has approximately 37% uronic acid, an equivalent weight of 511, and a measured molecular weight of 9,500,00 Daltons. It occurs as the calcium and magnesium salt. Hydrolysis produces D-Galactose, D-Glucronic acid, and L-rhamnose, units of which may be components of the main chain and several oligosaccharide fragments of the molecule, such as 2-O-α-D-galacturonoyranosyl -l-rhamnose,4-O-D-Galacturonopyranosyl-D-galactose and ß-D-glucuronopyranosy(1→3)-α-galacturonopyranosyl-l-(1→2)-l-rhamose.[8-10] Analysis[11,12] of various Indian and African gums show that they are quite similar in compostion. The molecular structure is still incompletely known.

Solution properties

Solubility – Gum karaya is one of the least soluble of the exudates gums. Particles in water do not dissolve but swell extensively. The texture of the water-gum mixture depends upon the degree of grinding, or particle size. Whereas coarse gum, when mixed with water, gives a grainy dispersion, a fine powder produces an apparently homogeneous dispersion. (To achieve a uniform dispersion with finely powdered gum karaya, one or more of the following procedures should be used; (1) applying vigorous agitation while adding the gum to water, (2) allowing two or more hours for hydration, (3) dispersing the gum into water as a mixture with another solid such as sugar, (4) premixing the gum with 1-5 times its weight of a water-miscible liquid, such as ethanol or glycerol, prior to the addition of water high initial rate of viscosity increase. The effect of particle size on viscosity development is shown by the fact that the rate of viscosity increase in a dispersion of 80-200 mesh gum Karaya is slower than that in a dispersion of a more finely powdered gum of the same quality. Normal viscosities of gum karaya dispersions range from about 400 cp for a 0.5% concentration to 10000 cp for a 3% dispersion. At pH values above 8, molecules lose their acetyl groups through rapid saponification, and dispersions become ropy and mucilaginous. At concentrations of 20% or more, gum karaya dispersions are adhesive and capable of forming strong bonds. Possibly because of the high uronic acid content, dispersions withstand acid conditions quite well.

pH – Gum Karaya is soluble at all pH values but has maximum solubility at pH 6-8; solubility decreases upon addition of acid or alkali. Higher viscosities and pH stability over a wider range are obtainable when the gum is hydrated prior to pH adjustment. The solution color lightens in acidic media and darkens in alkaline solutions because of the presence of tannins. At pH 7 and above, the characteristic short-bodied gum karaya solutions become ropy mucilages. This irreversible transformation, which has been described to deacetylation, is accompanied by an increase in viscosity. Gum karaya undergoes hydrolysis slowly and resists hydrolysis in 10% HCl solutions at room temperature for at least 8 hours. Acid concentrations of 4% at 50 °C for 10-24 hr or 90% for 1-2 hr are required to hydrolyze a solution to water thinness.

Temperature – When the temperature of a fully hydrated gum karaya solution is gradually raised from 20 to 85°C, the viscosity decreases. Boiling reduces the viscosity of gum karaya solutions, particularly when they are held at this temperature for more than 2 min, Higher maximum viscosity is obtained by cold hydration of gum karaya than is afforded by hot hydration. The reduction in viscosity that is obtained by cooking gum karaya suspensions, especially under pressure, is accompanied by an increase in the solubility of the gum. Under these conditions, it forms a smooth, homogeneous, translucent, colloidal dispersion. Concentration as high as 15-18% can be prepared in this manner.

Concentration – In dilute solutions of gum karaya, the viscosity increases linearly with concentration up to about 0.5%. Thereafter, gum karaya dispersions behave as non-Newtonian solutions.

Ionic strength – The viscosity of gum karaya dispersions decreases when electrolytes, such as sodium, calcium, an daluminum chlorides and aluminum sulfate, are added. When gum karaya is hydrated in solutions containing as much as 25% of a strong electrolyte, the viscosity is stable, although there is an initial depression that is accompanied by a separation of solids from solution. The normal viscosity of 0.5% high grade gum karaya solution (400 cp) drops below 100 cp when electrolytes are added. Sensitivity of gum karaya to strong electrolytes begins at low salt concentrations; the gum is not as sensitive to solutions of weak electrolytes.

Age of solution – The viscosity of gum karaya suspensions is stable over short periods but long term stability can be provided by the addition of preservatives, such as chlorinated phenols, formaldehyde, mercuric salts, and benzoic or sorbic acid. Gum karaya loses viscosity forming ability when stored in the dry state; the loss is greater for a powdered material than for the crude gum. Cold storage inhibits degradation. It has been suggested that the decrease in viscosity is related to the lose of acetic acid.

Gels

Gum karaya forms heavy, non-flowing pastes at concentrations above 2-3%. Its dispersions do not pass through a noticeable gel stage although they exhibit the absorption characteristics of gels, especially at high concentrations.

Films

Gum karaya forms smooth films. When plasticized with compounds such as glycols, it finds use in hair-setting preparations. However, practically all other applications of the gum depend on its viscosity and water-absorbing characteristics.

Applications

Pharmaceuticals – As a bulk laxative, it is second in importance to psyllium seeds. For this purpose, it is ground to 8-30 mesh. These coarse gum particles absorb water and swell to 60-100 times their original volume, forming a discontinuous type of mucilage that is very effective as a laxative. The gum is not digested nor is it absorbed by the body. Recent work shows that it is nontoxic and non-metabolized. Another important pharmaceutical application of gum karaya is as a denture adhesive. The powdered gum is usually dusted on the dental plate and swells when it touches the moist surfaces of the mouth. This results in a more comfortable and tighter fit of the plate. The rapid swelling of the gum particles, their relative insolubility, and their unusual resistance to bacterial and enzymic breakdown make the gum suitable for this use.

Paper – Gum karaya is used as a binder in long fibered, lightweight papers. For example, condenser tissues and fruit wrap tissues. Its effectiveness is due to removal of its acetyl groups by treatment with alkali, thereby exposing more active carboxyl and hydrocyl groups and increasing the polarity of the molecule. Removal of acetyl groups also affords an internally conhesive or ropy solution. To remove acetyl groups, a gum dispersion is placed with water in a wooden or stainless steel tank with high agitaition to thoroughly disperse the gum. Difficulty in dispersion can be overcome by the use of special dispersible gums. The gum is hydrated by allowing the dispersion to stand at room temperature for several hours or by heating it to 71-82 °C for 15 min. A weak alkali, such as ammonia or triethanolamine, is then added to raise the pH of the solution from its normal pH 4.5 to 8.0-8.5. The properties that are imparted to the gum by deacetylation are pH irreversible and are maintained even when the solution is added to an acidic pulp suspension.

Foods – Powdered gum karaya is used in French dressing, ice pops, sherbets, cheese spreads, ground meat preparations, and meringue products. In French dressing, it is used as a stabilizer. It is used sometimes in conjunction with gum Arabic, which acts as a protective colloid. Gum karaya is used in precnetagess of 0.2-0.4 in the manufacture of ice pops and sherbets. The gum prevents the

bleeding of free water and the formation of large ice crystals. Its water absorbing and holding capacities and its excellent acid compatibility make it suitable for this use. Concentrations of 0.8% or less of the gum are used in cheese spreads. Its acidic nature is not objectionable in this type of dairy product. It is added to prevent water separation and to increase the ease of spreading.

Textiles – Gum karaya is modified so that it can be used for printing operations in which it was considered unsatisfactory until a method of increasing its solubility was developed. This done by cooking a water suspension of gum karaya under pressure. The rate of dissolution varies with pressure. In commercial particle, solutions containing 15-18% of solids are marketed as textile gum solutions. An alternative method of solubilization consists of treatment with sodium peroxide, persulfate, or persilicate. The textile gum solution is used principally as a thickening agent for the dye in direct color printing on cotton fabrics.

MATERIALS AND EQUIPMENTS

Raw Materials

- Dried grinded powder of Samrong

Equipments

1. Hot air oven
2. Sieve mesh
3. Dryer
4. Vacuum pump

Chemical reagents

1. Ethyl alcohol
2. NaOH
3. HCl
4. K_2HPO_4
5. Na_2HPO_4
6. K_3PO_4
7. NaCl

EXPERIMENTAL DESIGN

Part I: STUDY ON THE EXTRACTION METHOD

Sample preparation

The dried whole *Sterculia lychnophora* Hance seed was broken and separated into the shell-mucilage part and the shell-endosperm part. The shell-mucilage part was grinded into powder and filter with sieves. The finished powder was stored in airtight container for further experiments.

1. To study the effects of pH on both swelling property and the extraction.

The basic concept of this experiment was based on the common extraction method of hydrocolloid. Seed was soaked and dissolved hydrocolloid into soluble form, then separated sediment or solid part. That soluble hydrocolloid was precipitated by adding two times of ethyl alcohol into solid form.

Ten grams of selected whole seeds was soaked with each 150-ml buffer solution of pH 2, 4, 7, 10, and 13 for 3 hours at room temperature. Each solution was observed and recorded the swelling properties. The solution was filtrated by passing through cheese cloth to remove the residues. Three times of ethyl alcohol were added into the filtrate. The precipitates were collected, and then dried on Petri Dish overnight. Weight of matter was recorded as yield.

2. To study the extraction method by NaOH

After the unsuccessful from the first experiment, this experiment was studied only the mucilage portion of Samrong seed and treated with NaOH solution.

Twenty-two grams of Samrong powder was weighted and boiled for three hours. Residue was filtered and added with 0.5 M NaOH overnight. Solution was adjusted to neutral pH and added two-times of ethyl alcohol to precipitate the hydrocolloid. The precipitated was washed with alcohol several times, dried in oven at 60°C for 20 min and grinded into powder. The yield and viscosity were recorded.

3. To study the effects of NaOH concentrations and heating on the extraction

This experiment was studied the effects of the NaOH concentration, HCl and heating on the yield and viscosity properties. The samples were divided into three groups as following:

3.1 The Boiling and NaOH Concentrations

Four sets of the five-grams seed powder was weighted and boiled. Each boiled mucilage was added into 150-ml solutions of 0.5 M, 1.0 M, 1.5 M, and 2.0 M. NaOH.

3.2 The Non-Boiling and NaOH Concentrations

Four sets of the five-grams seed powder was weighted and added directly into 150-ml of 0.5 M, 1.0 M, 1.5 M, and 2.0 M. NaOH.

3.3 The Boiling and HCl Concentrations

A 5-grams seed powder was weighted and boiled. The boiled-mucilage was added into 150-ml of 0.5M HCl.

All samples were adjusting to neutral pH and added two times of ethyl alcohol to precipitate the crude hydrocolloid. The precipitated was washed with alcohol several times, dried in oven at 60°C for 20 min and grinded into powder. The yield and viscosity were recorded.

4. The modified extraction method considering both quality and quantity

This experiment was concerned the effect of alkaline ageing which may be affect on the quality of the extracted powder. The experiment aimed to produce a higher quality of extracted powder.

Five-grams seed powder was weighted and added into boiled water for 3 hours. Residue was filtered and added with 0.5 M NaOH for 2 hours and then adjusted to pH 6. Two times of ethyl alcohol was added to precipitate out the gum, dried in the oven and then grinded. The percent of yield was recorded.

PART II: STUDY ON THE CONDITION THAT THE GUM CAN PROVIDE VISCOSITY.

A. Effect of pH I

0.72 g of the wet extracted gum from the experiment 2 (part I, 0.5M NaOH) was added into 30-ml buffer solution of pH 7, 8, 9, 10, and 11. The viscosity of samples was recorded.

B. Effect of pH II

0.25 g of the extracted powder from the experiment 3 (part I, 0.5 M of NaOH) was added into 20-ml buffer solution at of pH 4, 7, 10, and 12. The viscosity of samples was recorded.

C. Effect of NaCl concentration

0.25 g of the extracted powder from the experiment 2 (part I), was added into 30-ml of 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M. NaCl at pH 10. The viscosity of samples was recorded.

D. Effects of temperature, mixing method and pH

(a) Effect of mixing temperature

The 0.25 g of extracted powder from experiment 2 (part I) was added into 30-ml of solution at pH 10 at difference temperature, 3°C, 28°C and 60°C. After completely dissolve, 0.3 M NaCl was added and mixed thoroughly. The viscosity of samples was recorded.

(b) Effect of pH

The 0.25 g of extracted powder from experiment 2 (part I) was added into 30-ml of solution at pH 7 and 10 at room temperature. The 0.3 M NaCl was added and then mixed thoroughly. The viscosity of samples was recorded.

(c) Effect of mixing method

Three mixing methods were designed to study the effect of mixing method: (1) Premix Samrong powder and NaCl before adding into solution, (S-N-D) (2) Add Samrong powder first, dissolve in solution and then add NaCl later (S-D-N) and (3) Add NaCl first, dissolve in solution and then add Samrong powder later (N-D-S).

All treatments contained 0.25 g of Samrong powder, 0.3 M NaCl at pH 10 and room temperature. All samples were measured yield and viscosity.

E. Determination of Experiment 3 (Part I)

All samples from the experiment 3 were measured the viscosity to evaluate the properly extraction condition. Each sample contained 0.25 g of extracted powder dissolve in 30-ml solution at room temperature (28°C) and 60°C. All samples were measured viscosity.

F. Determination of Experiment 4 (Part I)

All samples from the experiment 4 were measured the viscosity to evaluate the properly pH after treating with NaOH in fallowing way: first sample at pH 6 and 0.85% of powder in distilled water (w/w); second sample at pH 6 and 0.5% of powder in distilled water (w/w); third sample at pH 2.6 and 0.85% of powder in distilled water (w/w). All samples were measured viscosity.

RESULT AND DISCUSSION

PART I

1. To study the effects of pH on both swelling property and the extraction.

Table 1: The appearances and yield of Samrong samples at various pH

Samples	Color	Swelling appearance	Yield (%)
pH 2	Light brown	Low swelling	1.99
pH 5	Brown	Moderate swelling	-
pH 7	Brown	Swelling very well	1.19
pH 10	Slightly dark brown	Swelling very well	3.168
pH 12	Dark brown	Jelly-like Form	-

The samples of pH 5 and 12 (table 1) couldn't measure the yield of extracted gum because they formed very sticky solution and difficult passed through the cheese clothes.

The extraction method of the Samrong gum was not successful in this experiment. The extracted gums of the rest samples were not able to grind into powder because it adhered on the Petri Dishes. The slightly swelling property was found at pH 2, and increased moderately at pH 5. It is obviously that the fully welling was achieved from pH 7 up to pH 12. This experiment concluded that pH was certainly affected on the swelling properties and the extraction method. The yield of gum in the experiment was lower than the expectation.

Jelly-like structure was found at pH 12 and shown the highly viscosity solution. The major problem was unable to dry the swelling mucilage into powder form. A new method should be further studied for modifying the swelling mucilage into the crude dried powder as mention in the aim of this project.

2. To study the extraction method by NaOH.

Table 2: The yield of crude hydrocolloid at 0.5 M NaOH

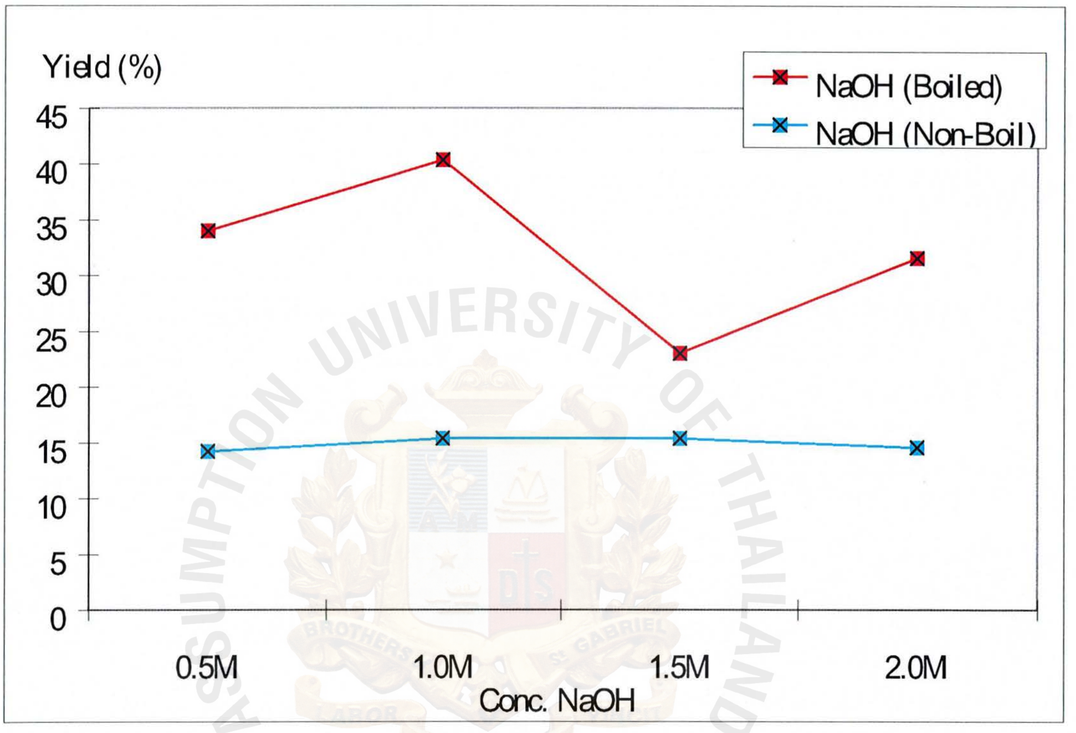
% Yield of crude hydrocolloid	17.18 %

The Samrong was grinded and prepared by discard the skin and other part that was no mucilage part. The first modification, boiling, was to fully swelling raw material. After boiling, the appearance of mucilage was like several bounded pellets. The second modification used NaOH as a solvent to soften and dissolve the pellets or the swelling mucilage. The mechanism might be explained that membrane of the swelling mucilage was broken and substance which expected as hydrocolloid was released into a semi-solid solution. After several times of filtrations, the filtrate then precipitated with alcohol. Fibers like substances which expected as hydrocolloid were precipitated out from the filtrate. The substances were dried first then weighted. The result was calculated into percentage of yield from the original 22 gram of prepared Samrong.

This experiment gave an ardent result that the extraction method to gain the expected hydrocolloid or non-starch gum powder. The yield of the extracted powder also was increased when compared with the first experiment. (table 2) This method should be the guideline in further the extraction of crude gum. The powder were further investigated which conditions to form viscosity in the experiment part II, section A and C.

3. To study the effects of NaOH concentrations and heating on extraction.

Graph 1: The yield of crude Samrong gum at different concentration of NaOH and heating effects



In this experiment, (graph 1) the extraction method of Samrong was following the second experiment as guild line. The samples were separated into boiling and without boiling the mucilage.

The appearance of the boiled mucilage was the same as pre-described like several bounded pellets with homogeneous texture. Without heating, the appearance of the non-boiled mucilage was not formed into pellets in the form of heterogeneous texture after soaking with NaOH. It might not uptake the water at all and fully swelling of mucilage.

The volume of the boiled mucilage was higher than the non-boiled mucilage in the ratio of 1.5:1 due to the uptake of water from the boiled one. The difference of solution level was added up by the distilled water.

The fiber like hydrocolloid was extracted from both NaOH solutions

by ethyl alcohol. The non-boiling can be extracted crude gum due to extend the soaking time for fully swelling. The crude gum was dried at 35°C within 30 minutes. The dry gum was then grinded into powder form by mortar. The percentage yield was calculated and recorded to compare with the previous experiment.
The yield of non-boil samples was obviously lower than the boiled one in all concentrations. Heating may change their pellet membrane or change in structure of polysaccharide due to fully hydrated after heating for 3 hours.

The yield of the extracted grinded powder increased greatly only in alkaline conditions. For overall, the percentage yield in this experiment was two times more than the second experiment. It might be result from the shorter soaking time with NaOH. It could be said that 1.0 M of NaOH solution gave better yield if only one factor – concentration of NaOH was considered.

No precipitate was found at 0.5 M HCl. Gum karaya undergoes hydrolysis slowly and resists hydrolysis in 10% HCl solutions at room temperature for at least 8 hours (Cornucopia II , 1998). The Samrong mucilage maybe hydrolyzed into a short chain molecule by HCl under high temperature.

The conclusion was that acid extraction was not suitable for the extraction. This project recommended crude Samrong gum should be extracted under alkaline condition. Due to the effect on viscosity property still not known and it would be carried out in the experiment part II. Before do the experiment four, all samples were evaluate the viscosity and will discuss in part II section.

4. The modified extraction method considering both quality and quantity

Table 3: The yield of hydrocolloid powder from new modified method

% Yield of crude hydrocolloid	37.94 %

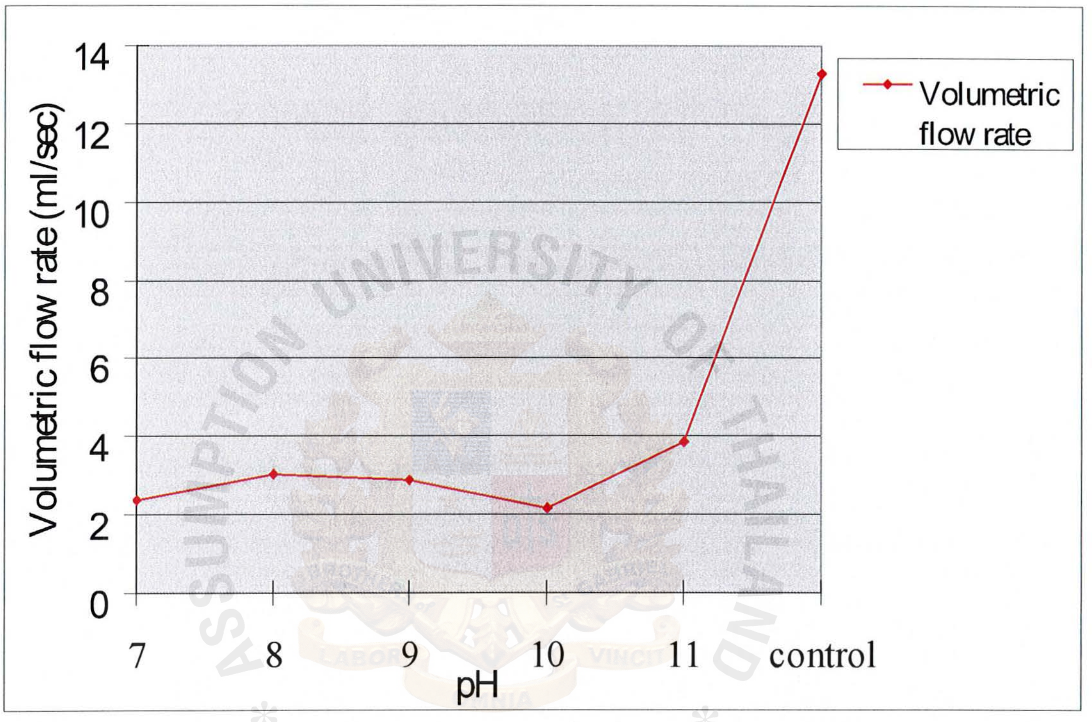
The yield in the second experiment was 17.18% (table 1) and in third experiment was around 35% (table 2) (boiled one), and in this experiment four was 37.94% (table 3). The yield was increased slightly but not much different from the third experiment. It means the optimum yield of Samrong may be 35% to 38% of grinded powder excluding the seed endosperm and shell.

By doing this experiment another factor – soaking time (with NaOH solution) that effect both quality and quantity of powder was considered. In this experiment, the modified extraction method gave a guide line about the extracted powder in both quantity and quality. The quality of Samrong would be studied and discussed in part II. In expectation, the mucilage contains 35% to 38% of hydrocolloid (polysaccharide).

Part II STUDY ON THE CONDITION THAT THE GUM CAN PROVIDE VISCOSITY.

A. Effect of pH I

Graph 2: The volumetric flow rate of crude gum in each pH buffer solution (powder from Part I section 2).



The testing of viscosity was carried out to examine the extracted powder with both effects of pH and NaOH concentration. The wet crude gum of all samples was not dissolved quite well until heating at 60°C. Both pH 7 and pH 10 were noted to be sticky in graph 2. The volumetric flow rate dropped at pH 10. The additional of salt (mono- or divalent) of buffer solution for adjusting pH might be influence on the viscosity behavior in this experiment. Distill water is used as a control to clear the error in this experiment. The control showed high volumetric flow rate which means there was no viscosity forming.

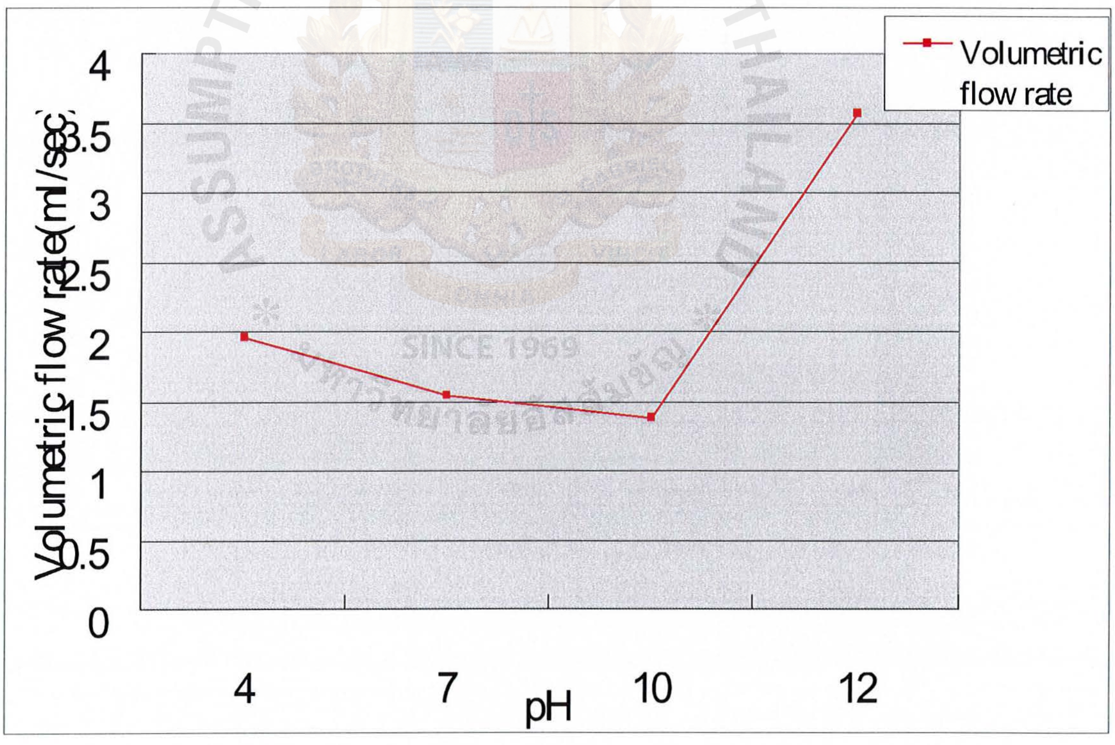
Gum Karaya has maximum solubility at pH 6-8 and solubility decreases upon addition of acid or alkali. Higher viscosities and pH stability over a wider range are obtainable when the gum is hydrated prior to pH adjustment. This mean that about pH 6 to 8, the solubility of the Gum karaya are the highest and the viscosity also achieve the optimum due to more gum disperse in the solution.

It was hardly to conclude that low solubility and viscosity might be similarly the gum Karaya because the extraction method and viscosity conditions were not fully complete understanding.

By the way, it could be conclude that the mucilage part contained the major part of non-starch gum. The pH should further study in the next experiment to conclude about the optimum pH.

B. Effect of pH II

Graph 3: The volumetric flow rate of crude gum in each pH buffer solution (powder from experiment part I section 3 (0.5 M NaOH)).

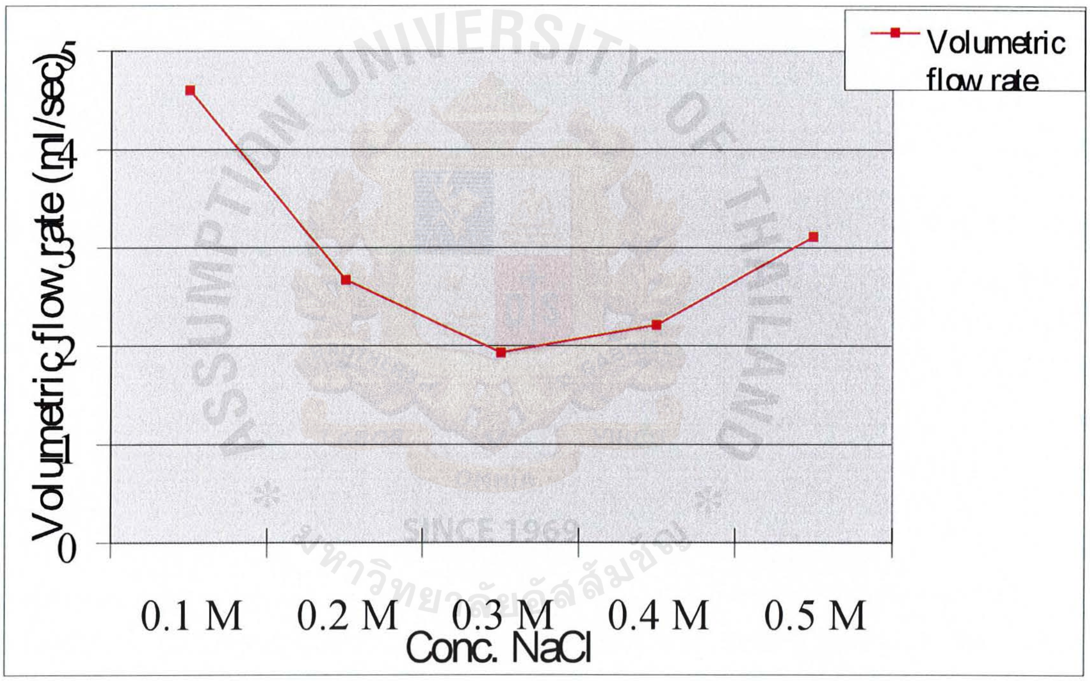


In this experiment, a various pH buffer were examined to guild the optimum pH (range) in leading the highest flow rate. The volumetric flow rate was decreased when solution below natural and increased above pH 10. Comparing the experiment A, both pH 10 gave the lowest volumetric flow rate and pH 7 gave the second lowest flow rate.

So it could be concluded that the solution for samples of Samrong powder should be neutral to slightly base. The pH has certain affects on the viscosity of the Samrong powder. Therefore, pH is the one of the main factor affects its quality. The other factors such as mono- or divalent salts and temperature will be examined in next experiment.

C. Effect of NaCl concentration

Graph 4: The volumetric flow rate of crude gum in different concentrations of NaCl at pH 10 from experiment 3 (0.5 M NaOH)

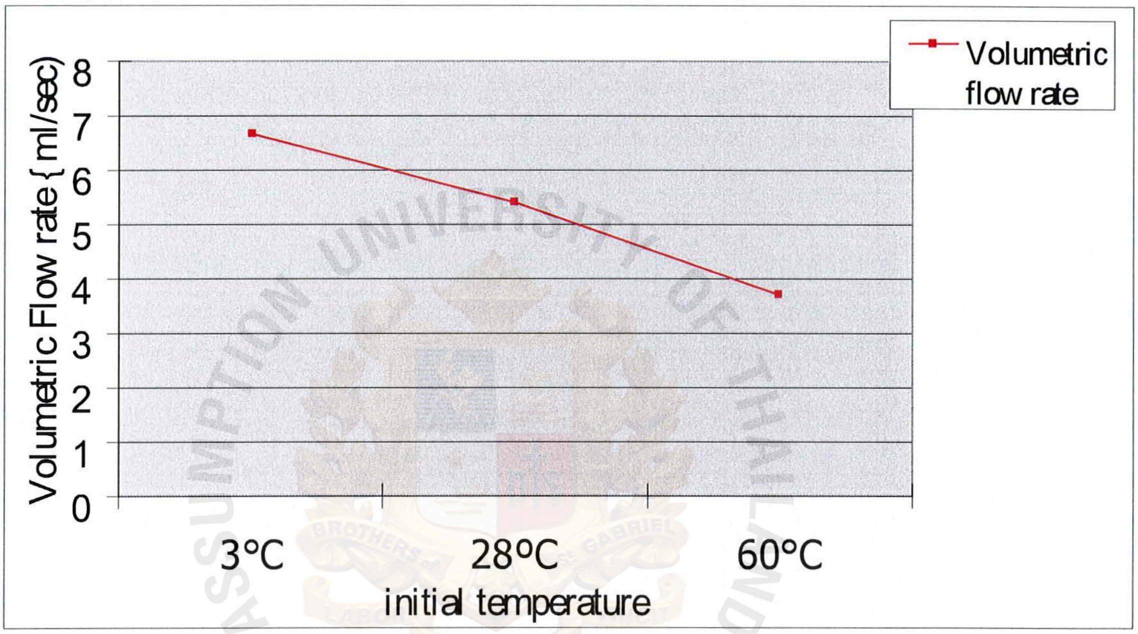


From graph 4, the concentration of NaCl had affect on viscosity formation. The viscosity was increased when the NaCl concentration was increased from 0.1 M to 0.3 M NaCl. The lowest flow rate was measured at 0.3 M NaCl then continues decreased from 0.4 M to 0.5 M NaCl.

D. Effects of temperature, mixing method and pH

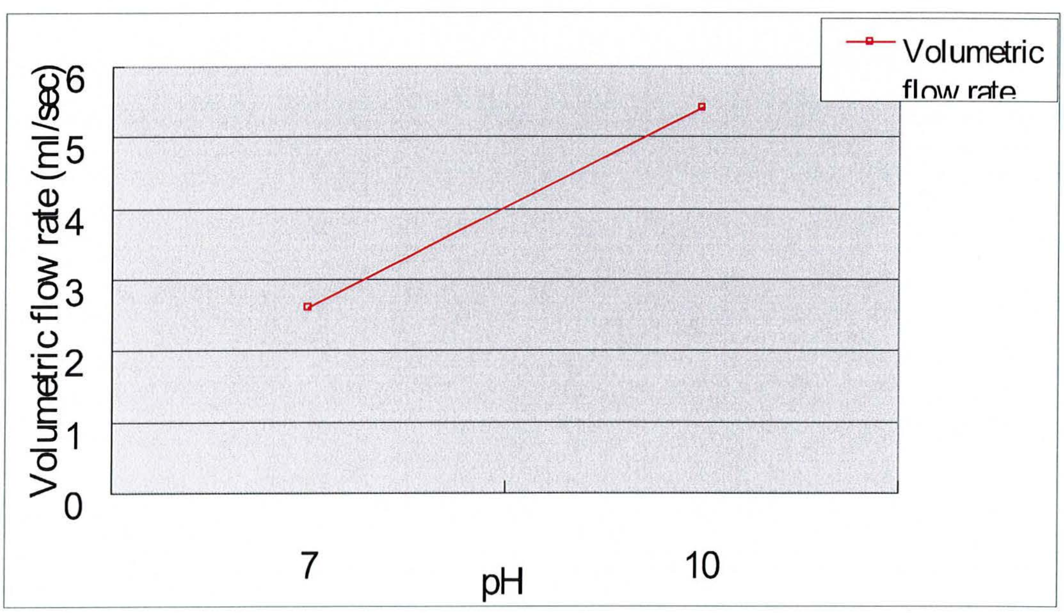
(a) Effect of mixing temperature

Graph 5: The volumetric flow rates of 0.25 g crude gum with different mixing temperature (powder from experiment 3., 0.5 M NaOH)



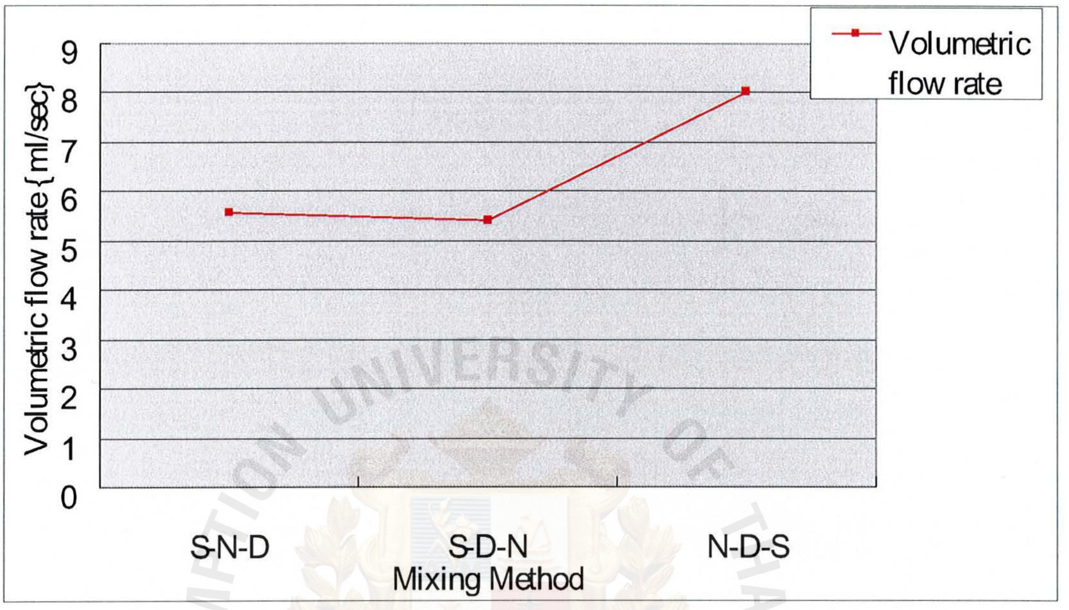
(b) Effect of pH

Graph 6: The volumetric flow rates of 0.25 g crude gum with different pH (powder from experiment 3, 0.5 M NaOH)



(c) Effect of mixing method

Graph 7: The volumetric flow rates of 0.25 g crude gum with different mixing method (powder from experiment 3., 0.5 M NaOH)



Due to the previous experiment Part II, Two groups of major factor affected on quality and quantity of crude Samrong gum – one group was extraction factors (ratio of powder to water, heating time and effect, soaking time, NaOH concentration, etc.) and another was viscosity factors (concentration, pH, mixing temperature, salt, mixing method, etc.) which both were not clearly understood.

This experiment tries to set up standard conditions for measuring the quality of crude gum at the extraction stage (experiment part I, sec. 3 and 4). The conditions from the previous in part II were uncertainly and unpredictable. The fluctuated results may be caused by difference in powder batches (part I, sec. 2 and 3) or interference between those factors mention above. Due to the small amount of samples and aims only find standard condition especially for this project. This experiment was designed to repeat all conditions with the powder sample from experiment 3.

In this experiment, the factors – temperature, pH and mixing method were studied to know how much they affect the flow rate (viscosity) of dissolved Samrong powder.
In (a), graph 5, three different temperatures- 3°C, 28°C and 60°C were prepared to study about the affect on flow rate. Higher temperature gave low volumetric flow rate.

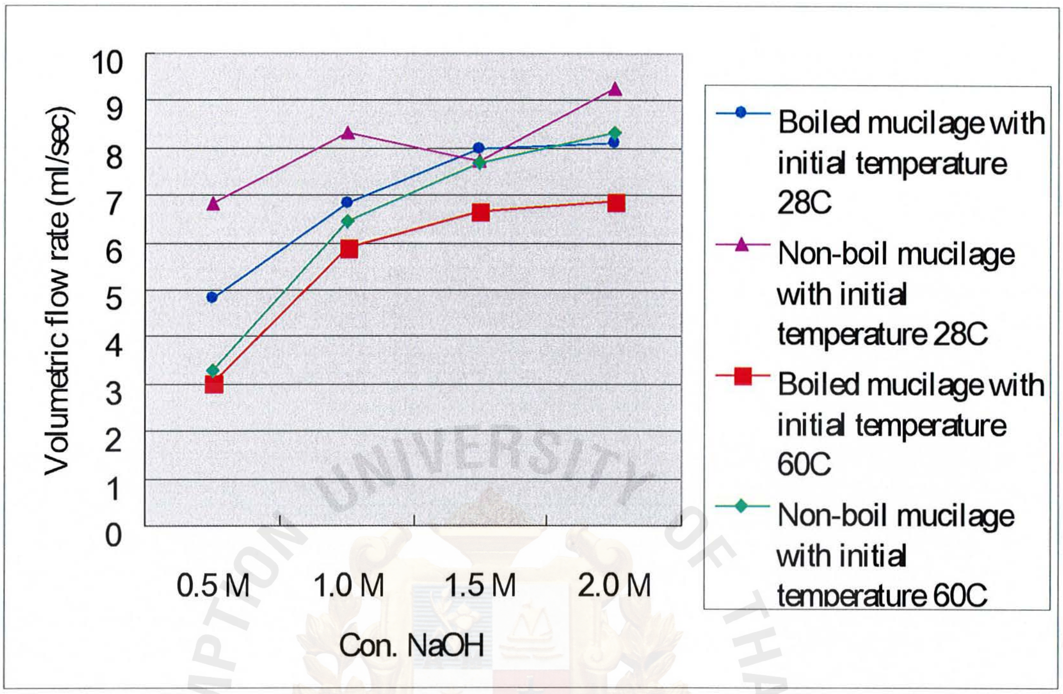
In (b), graph 6, two different pH obtained from experiment B were examined again. pH 7 gave a lower volumetric flow rate then pH 10

In (c), graphs 7, three order of mixing methods were examined. The result showed that the order of adding sample powder first or adding sample powder together with mono-valent salt (NaCl) had no different. But the result dropped when salt was added first. The sample was difficult to be dissolved in the solution which salt was added first.

From those above result, standard conditions should be in the step that 0.25 g. crude powder dissolve in distill water (~pH 7) and slowly heat up to 60°C for experiment E and F.

E. Determination of Ex 3.

Graph 8: The volumetric flow rate in each conc. Of NaOH solution



From graph 5, the viscosity formation was observed clearly from the concentration of 0.5M to 1.0 M NaOH. If extracted method use over than 1.0 M NaOH, flow rate was no difference in both boiling and heating effects. The higher concentration of NaOH may destruct gum structure then result in the decreasing of solubility and a lower grade of gum.

The graph above showed that the highest flow rate was lined on the B-B (the boiled mucilage in 0.5 M with the initial temperature 60°C). The result was dropped dramatically. So the mucilage had to be boiled before adding into NaOH solution. The higher viscosity had gain after fully dissolve gum by heating in both N-B (the non-boiled mucilage with the initial temperature 60°C) and B-N (the boiled mucilage with the initial temperature 28°C). The lowest viscosity (higher volumetric flow rate) was found in non heating samples, N-N (the non-boiled mucilage with the initial temperature 28°C). it may assume that viscosity formation of gum was thermal dependent.

The higher viscosity (lower volumetric flow rate) had gain at 0.5 M NaOH and at least heating one time especially dissolves stage.

F. Determination of Experiment 4 (Part I)

Table4: The Volumetric flow rate of prepared pH and the concentration of sample from experiment four.

pH of the solution	Concentration of solution (%)	Volumetric flow rate (ml/sec)
6	0.85	0.05
6	0.5	0.18
2.6	0.85	0.19

In this experiment, the extraction method was modified into the final stage. The samples were prepared as usual, and the testing of flow rate was carried out to examine the viscosity of the extracted powder in this batch. The first sample (pH 6 and 0.85%) gave a significant flow rate over the other previous experiment. The second sample (pH 6 and 0.5%) was carried out to test the minimal dose of powder added in solution. the third sample (pH 2.6 and 0.85%), the idea of this testing was decolorizing the powder into light brown color using acid. According the result in the third sample, the flow rate was dropped gradually comparing the same dose of powder in sample number one.

From the result, the higher viscosity had gained by higher concentration of gum (0.85% > 0.5%) or higher pH (pH 6 > 2.6)

The extracted method in this experiment was modified by using the optimum factors (boiling mucilage, 0.5 M of NaOH, soaking time, pH of neutralization, concentration of gum, and pH and heating of dissolve stage). The grade of extracted powder from this experiment was the better in both yield and flow rate (which relate to viscosity).

CONCLUSION

Factors affect the yield of extraction method as following:

- Temperature: boiling mucilage for three hours
- Concentration of NaOH: 0.5 M or 1.0 M
- Neutral pH: pH 6-7
- Soaking time: one hours
- Filtration: Filtering impurity before precipitation for more then five times

Factors affect the viscosity formation as following:

- Temperature: heating at 60 °C after fully dissolve
- pH solution: neutral pH
- Concentration of gum: 0.85%

When the extracted Samrong powder mixed with the buffer, it formed a very viscous solution under varies pH buffers. Although it was very viscous and looked like gel, it could not set or maintain its shape. It is possible for extracted Samrong powder to be a thickening agent. The condition(s) that make Samrong powder form gel are still not clear. It needs more deep research in the structure of Samrong and factors effect its gelation.

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