CHAPTER III

EXPERIMENTAL METHODS AND MATERIALS

3.1 Apparatus

3.1.1 Composition analysis

3.1.1.1 Moisture content

3.1.1.1.1 Aluminium dish with cover

3.1.1.1.2 Electric air oven

3.1.1.1.3 Desiccator with silica gel

3.1.1.1.4 Analytical balance

3.1.1.2 Ash

3.1.1.2.1 Porcelain dish or crucible

3.1.1.2.2 Electric muffle furnace

3.1.1.2.3 Electric air oven

3.1.1.2.4 Desiccator with silica gel

3.1.1.2.5 Analytical balance

3.1.1.3 Fiber

3.1.1.3.1 Beaker with no lip 600 ml.or glass bottle fordigestion

3.1.1.3.2 Digestion apparatus with condenser

3.1.1.3.3 Linen cloth (size 200 mesh)

3.1.1.3.4 Gooch crucible or alundum crucible R-98

3.1.1.3.5 Electric air oven

3.1.1.3.6 Electric muffle furnace

3.1.1.3.7 Desiccator with silica gel

3.1.1.3.8 Analytical balance

3.1.1.4 Protein

3.1.1.4.1 Macro-Kjeldahl digestion flask

3.1.1.4.2 Distillation apparatus

3.1.1.4.3 Burette

3.1.1.4.4 Erlenmeyer flask 500 ml.

3.1.1.4.5 Dropper

3.1.1.4.6 Heater

3.1.2 Extraction process

3.1.2.1 Beaker 600 ml, 1,000 ml

3.1.2.2 Glass funnel

3.1.2.3 Linen cloth

3.1.2.4 Hot plate

3.1.2.5 Mechanical stirrer

3.1.2.6 Thermometer, 0-100 ^{o}C

3.1.2.7 Blender or scissors

3.1.2.8 Stand with clamp

3.1.2.9 pH meter and pH-paper

3.1.3 Bleaching

3.1.3.1 Beaker 600 ml

3.1.3.2 Stirring rod

3.1.3.3 Hot plate

3.1.3.4 Suction flask

3.1.3.5 Suction funnel

3.1.3.6 Vacuum pump

3.1.3.7 Filter paper

3.1.4 Alkali pretreatment

3.1.4.1 Beaker 1000 ml

3.1.4.2 Heater

3.1.4.3 Thermometer, 0-100 ^{o}C

3.1.5 Testing of the property of agar

3.1.5.1 Gel strength

3.1.5.1.1 Beaker 1000 ml

3.1.5.1.2 Stand with clamp

3.1.5.1.3 Burette

3.1.5.1.4 Plunger or Cylindrical glass rod, x-section area = 1 cm^2

3.1.5.1.5 Plastic clay

3.1.5.2 % Transmittance

3.1.5.2.1 Spectrophotometer (Bausch & Lomb, Spectronic 20)

3.1.5.2.2 Bausch & Lomb 1-cm cells

3.1.5.3 Rate of dissolution, Rate of gel forming, Gelation temperature and Gel melting Temperature

3.1.5.3.1 Stop watch

3.1.5.3.2 Thermometer, 0-50 ^{o}; 0-100 ^{o}C

3.1.5.3.3 Heater

3.1.5.3.4 Beaker 600 ml

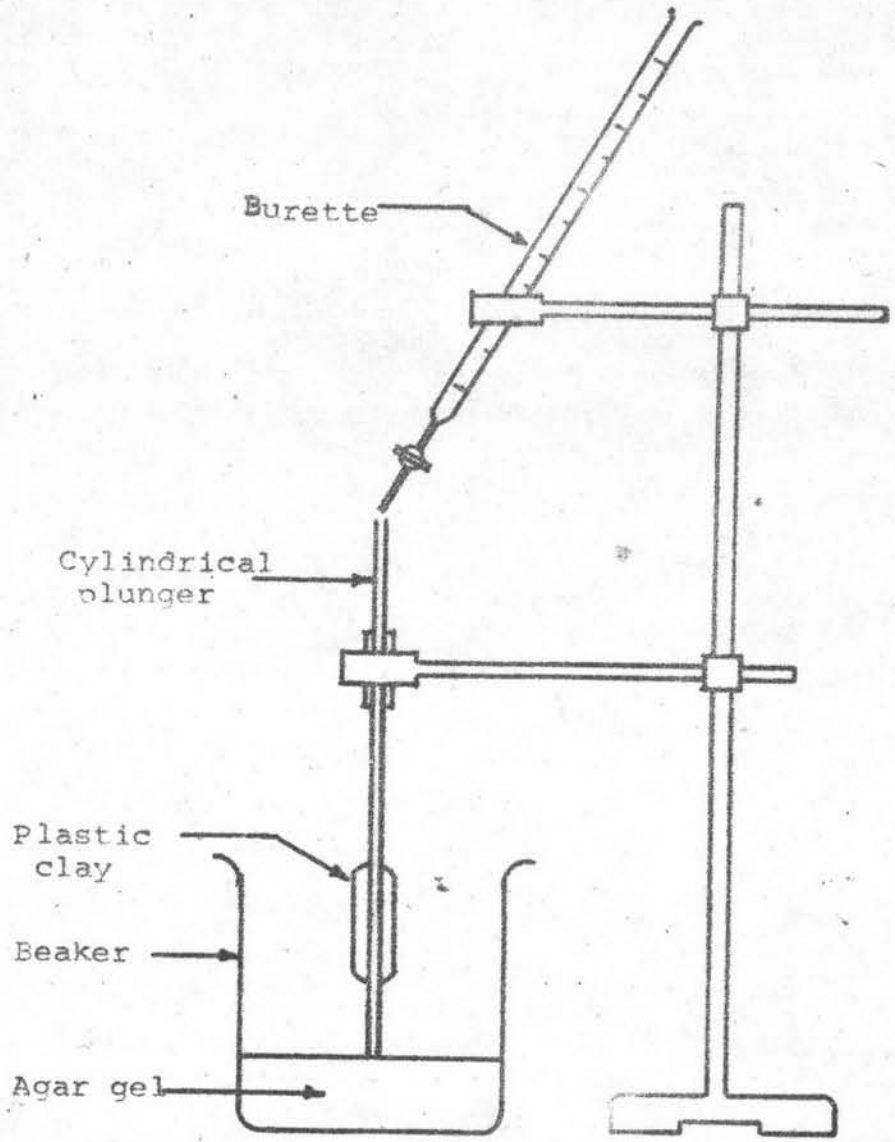

Figure 3-1 Gel strength

Apparatus for measuring the Gel strength

3.2 Materials and Chemicals

3.2.1 Composition analysis

3.2.1.1 Fiber

3.2.1.1.1 0.255 N Sulfuric acid

3.2.1.1.2 0.313 N Sodium hydroxide

3.2.1.1.3 95% Ethanol

3.2.1.2 Protein

3.2.1.2.1 Catalyst (Murcuric oxide, HgO and Copper sulfate, $CuSO_4 \cdot 5H_2O$)

3.2.1.2.2 45% Sodium hydroxide

3.2.1.2.3 0.2 N Sodium hydroxide

3.2.1.2.4 8% Sodium thiosulfate

3.2.1.2.5 0.5 N Hydrochloric acid

3.2.1.2.6 Methyl red (1 gm in 200 ml alcohol)

3.2.2 Extraction

3.2.2.1 Sundried seaweed from southern Thailand

3.2.2.2 1% Sulfuric acid

3.2.2.3 Acid sodium phosphate buffer, pH = 5.5

3.2.3 Alkali pretreatment

3.2.3.1 Sundried seaweed from southern Thailand

3.2.3.2 1% Sodium hydroxide

3.2.3.3 Dilute hydrochloric acid

3.2.3.4 Distilled water

3.2.4 Bleaching

3.2.4.1 Activated charcoal

3.2.5 Washing

3.2.5.1 Acetone

3.2.5.2 Ethanol

3.2.5.3 Distilled water

3.3 Experimental Methods and Procedures

3.3.1 Methods of Chemical Analysis

3.3.1.1 Moisture content

i) Dry the aluminium dish with cover (3.1.1.1.1) in the oven (3.1.1.1.2) at 105°- 107°C for 15 minutes.

ii) Replace the lid and place the dish in the desiccator (3.1.1.1.3) to cool for 30 minutes

iii) Weigh the dish with the cover to the fourth decimal place (3.1.1.1.4)

iv) Weigh the sample (seaweed) in the dish with the cover to the fourth decimal place of 5 gm.

v) Transfer the dish to the oven

vi) After 2 hours in the oven, replace the cover, and transfer the dish to a desiccator, allow to cool and weigh.

vii) Return dish to the oven and heat again for each 30 minutes, and weigh as before. Loss of weight between successive weighings should not exceed 2 mg.

viii) Calculate the percentage of moisture content as follow.

$$\text{Percentage of moisture content} = \frac{100\ (W_1 - W_2)}{W_1 - W}$$

When W = Weigh of aluminium dish with cover, gm

W_1 = Weigh of aluminium dish with cover and sample before dry, gm

W_2 = Weigh of aluminium dish with cover and sample after dry, gm

3.3.1.2 Ash

i) Ignite porcelain dish (3.1.1.2.1), cool in the desiccator (3.1.1.2.4) and weigh (3.1.1.2.5)

ii) Weigh 5 gm of the seaweed sample in the dish to the fourth decimal place.

iii) Burn gently over low flame until thoroughly charred.

iv) Transfer to muffle (3.1.1.2.2) at 600 $\pm$ 2°C and burned to a white or pale grey ash (about 2-3 hr)

v) Transfer to desiccator and allow to cool. Moisten the ash with water, re-ignite and weigh.

vi) Repeatedly return dish to the muffle and ignite again for 30 min until loss of weigh between successive weighing do not exceed 1 mg.

vii) Calculate the percentage of ash as follow.

$$\text{Percentage of ash} = \frac{100\ (W_2 - W)}{W_1 - W}$$

When W = Weigh of porcelain dish, gm

W_1 = Weigh of porcelain dish and sample before ignite, gm

W_2 = Weigh of porcelain dish and sample after ignite until constant weight is obtained, gm.

3.3.1.3 Fiber

i) Weigh 2.5 gm sample and mix with 200 ml of 0.255 N H_2SO_4 (3.2.1.1.1) in a beaker (3.1.1.3.1)

ii) Transfer to digestion apparatus (3.1.1.3.2) and digest for 30 minutes

iii) The solution was filtered through filter cloth (3.1.1.3.3). Wash the residue with 200-300 ml hot water.

iv) The residue was transfered to the beaker and 200 ml of 0.313 N NaOH (3.2.1.1.2) was added. Digest again for 30 minutes.

v) The solution was filtered immediately through Gooch crucible (3.1.1.3.4) and washed with hot water.

vi) The residue was washed again with 10 ml ethyl alcohol (3.2.1.1.3)

vii) Then it was dried in oven (3.1.1.3.5) at 105-110°C for 3 hrs and weighed

viii) Transfer the dried material obtained to the muffle (3.1.1.3.6) and ignite until fiber-free at 600°C (approx 30 minutes). Reweigh.

ix) Calculate the percentage of fiber as follows:

$$\text{Percentage of fiber} = \frac{100\ (W_1 - W_2)}{W}$$

Where W = Weight of sample, gm

W_1 = Weight of crucible and residue after dry, gm

W_2 = Weight of crucible and ash after ignite, gm

3.3.1.4 Protein

The Kjeldahl nitrogen was determined by methods of AOAC (21). Protein was calculated from the amount of nitrogen multiplied by 6.25

i) Weigh 2.5 to 3.0 gm sample.

ii) Weigh 0.85 gm (0.7 gm HgO + 0.15 gm $CuSO_4 \cdot 5H_2O$)(3.2.1.2.1).

iii) Mix i) and ii) in macro-Kjeldahl digestion flask (3.1.1.4.1) and digest until a clear solution was obtained (about 2.5-3.0 hrs.)

iv) The clear solution was diluted with 200 ml of water and distilled with a mixture of 25 ml of 45% sodium hydroxide (3.2.1.2.2) and 50 ml of 8% sodium thiosulfate solution (3.2.1.2.4).

v) Distill in distillation apparatus (3.1.1.4.2) until the distillate in a receiving flask containing initially 50 ml of 0.5N hydrochloric acid (3.2.1.2.5) attained a total volume of about 200 ml.

vi) Add 4 drops of methyl red (3.2.1.2.6) as an indicator.

vii) Titrate the distillate with 0.2N sodium hydroxide (3.2.1.2.3) to give a yellow end point.

3.3.2 Method of Extraction

Diagram of extraction process is shown in Figure 3-2(22) and the procedure is as follows:

i) Weigh 10-20 gm seaweed and wash with water about 2-3 times to remove any sand and epiphytes.

ii) Blend the seaweed in a blender (3.1.2.7) or cut into small pieces with the scissors (3.1.2.7).

iii) Boil the seaweed in the beaker (3.1.2.1) with 250-500 ml distilled water. Control the acidity either by 1% H_2SO_4 (3.2.2.2) or acid sodium phosphate buffer to pH = 5.5-6.5 and stir with mechanical stirrer (3.1.2.5) at speed No 10 for 2 hrs.

iv) Filter through linen cloth (3.1.2.3) and then clarify with 1% activated carbon (3.2.4.1)

v) After filtering by pressure filter (3.1.3.4-7), the filtrate was allowed to cool and set at room temperature.

vi) Transfer the gel to refigerator at temperature below-10^{o}C and left overnight.

vii) Thaw and dewater

viii) Repeat freezing and thawing for about 2-3 times

ix) Then the agar was washed with acetone, ethanol and dried in oven.

3.3.3 Method of Alkali Pretreatment (23)

i) Wash the seaweed 2-3 times with water

ii) Treat the seaweed with 1% NaOH (3.2.3.2) in a

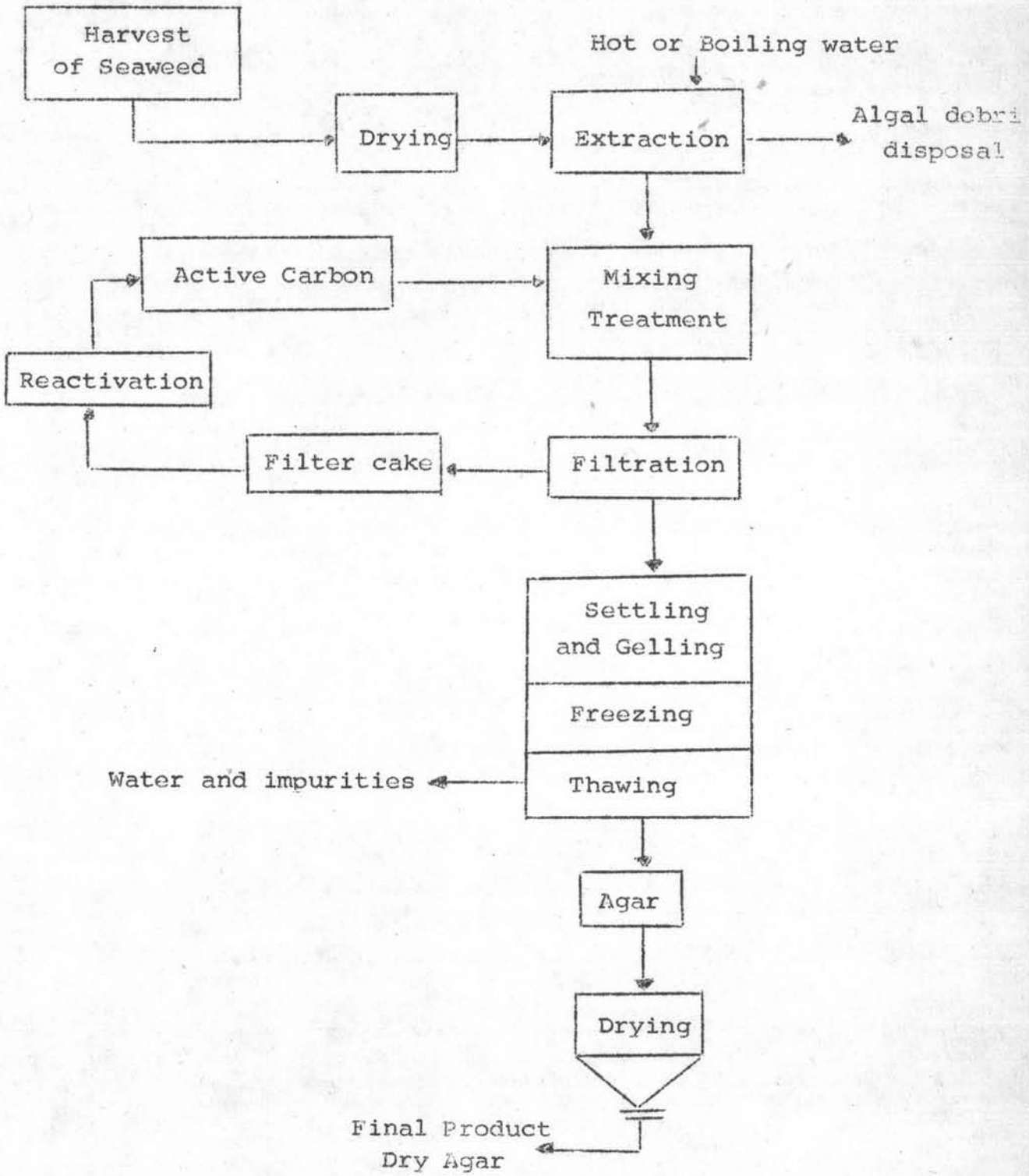

Figure 3-2 Agar production from seaweed

beaker (3.1.4.1) at 90°C for about 2 hrs.

iii) Then neutralize with dil HCl (3.2.3.3) and wash with distilled water (3.2.3.4).

Diagram showing the alkali pretreatment of seaweed before extraction is shown in Figure 3-3.

3.3.4 Determination of the Optimum Conditions for Extracting agar from seaweed

3.3.4.1 Effect of pH of solvent

i) Weigh 4 samples of 10 gm each

ii) Carry out the extraction according to method of extraction (3.3.2). Each sample was extracted with 250 ml of solvent.

-sample 1 was extracted with distilled water at pH = 6.9

-sample 2 was extracted with distilled water + 1%H_2SO_4 at pH = 5.5

-sample 3 was extracted with distilled water + 1%H_2SO_4 at pH = 5.5 and 1% H_2SO_4 was slowly added to control the pH of the solution during extraction at between pH = 5.5-6.5.

-sample 4 was extracted with 1% acid sodium phosphate buffer at pH = 5.5

iii) Calculate the percentage yield of each sample

3.3.4.2 Effect of time

i) Weigh samples of 10 gm each.

ii) Samples were extracted with various times

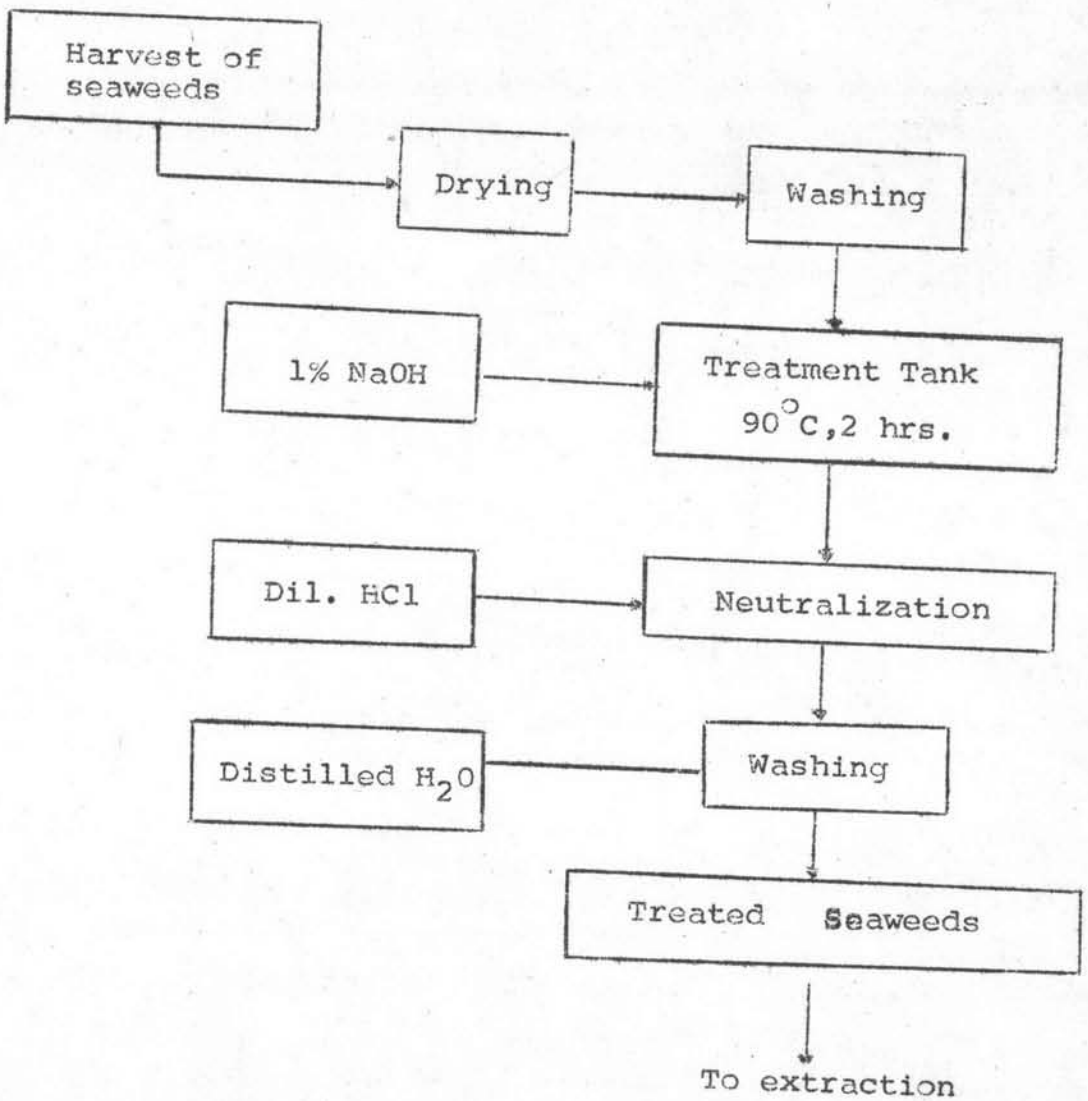

Figure 3-3 Alkali pretreatment of seaweed before extraction.

viz. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hrs. respectively. The extraction was done by method of extraction (3.3.2) using 250 ml of acid sodium phosphate buffer, pH = 5.5 as solvent.

iii) Calculate the percentage yield of each sample.

3.3.4.3 Effect of amounts of solvent used

i) Weigh 6 samples of 10 gm each.

ii) Samples were extracted with various amounts of solvent from 100 ml to 500 ml. The extraction was done by method of extraction (3.3.2) using acid sodium phosphate buffer, pH = 5.5 as solvent.

iii) Calculate the percentage yield of each sample.

3.3.5 Effect of Alkali Pretreatment on % Yield

i) Each sample was divided into 2 parts

ii) The first part was treated by method of alkali pretreatment (3.3.3) but the second part was not.

iii) Each part was extracted by method of extraction (3.3.2) under optimum conditions.

iv) Calculate the percentage yield of each sample.

3.3.6 Determination of the % Yield of Agar Extracted from Seaweed from Various Sources in Southern Thailand.

i) Seaweeds used in extraction were obtained from

3 sources in southern Thailand : Ranong, Songkla and Surat-thanee.

ii) All seaweeds were treated by method of alkali pretreatment (3.3.3) before extraction.

iii) Extraction was done by method of extraction (3.3.2) under optimum conditions.

iv) Calculate the percentage yield of each sample.

3.3.7 Method of Testing the Property

3.3.7.1 Gel strength (24)

i) Weigh 1.5 gm dried agar and dissolve in 100 ml boiling water in a 1,000 ml beaker (3.1.5.1.1).

ii) Allow to cool at room temperature for 2 hrs

iii) Set the equipment as shown in Figure 3-1

iv) After 2 hrs setting, test the agar gel with the equipment shown in Figure 3-1 (1).

v) Press a 1-cm^2, cylindrical plunger into the gel without lateral movement for 20 sec. Plastic clay was used as loading weight. In addition, water can be added in the plunger from burette above.

vi) Record the volume of water used from burette that required to effect rupture in 20 sec.

vii) Weigh the 1-cm^2, cylindrical plunger together with the plastic clay used.

viii) Calculate the gel strength of agar gel as follows:

$$\text{Gel strength in gm/cm}^2 = \frac{W + P + L}{A}$$

where W = Weigh of water used in plunger

P = Weigh of plunger

L = Weigh of loading weight (plastic clay)

A = X-sectional area of plunger.

3.3.7.2 % Transmittance (25)

i) Prepare 1.5% agar solution

ii) Transfer the hot 1.5% agar solution in a Bausch & Lomb 1-cm cell (3.1.5.2.2). Distilled water at the same temperature as agar solution was used as blank.

iii) Measure the % transmittance of 1.5% agar solution at 520 mμ using a Bausch & Lomb, Spectronic 20, Spectrophotometer (3.1.5.2.1).

3.3.7.3 Rate of dissolution

i) Weigh 1.5 gm dried agar

ii) Prepare 100 ml hot distilled water at 90-100°C in a beaker (3.1.5.3.4)

iii) Record the time with a stopwatch (3.1.5.3.1) after adding agar in hot water until the clear solution was obtained.

3.3.7.4 Rate of gel forming and gelation temperature

i) Record the time after standing the clear solution of 1.5% agar solution (3.3.7.3) in room temperature until it began to gel.

ii) Record the temperature when it just began to gel

3.3.8 Effect of Alkali Pretreatment on Some properties of Agar Extracted from Seaweed

i) Test the properties of agar extracted by alkali pretreatment and without alkali pretreatment by methods of testing the property (3.3.7.1-3.3.7.5)

ii) Determine the moisture content in each agar by Methods of Chemical Analysis (3.3.1.1).

3.3.9 Determination of Some Properties of Agar Extracted from Seaweed from Various Sources in Southern Thailand

i) Dried agar obtained from (3.3.6) were tested by methods of testing the property (3.3.7.1-3.3.7.5)

ii) Determine the moisture content in each agar by methods of Chemical Analysis (3.3.1.1).