Laboratory test procedures

for raw-dried seaweed and semi-refined carrageenan from Eucheuma and Kappaphycus

SEAPlant.net Monograph no. HB2H 1008 V3 LTP

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INTRODUCTION

Much of the material presented here originally appeared in "*The ABC of Eucheuma Seaplant Value Chains*" by Iain C. Neish (SuriaLink Monograph No. 1-0104 - ISBN 983 2893 03 8). That monograph is now out of print and it is being supplanted by the present HB2 series of SEAPlant.net monographs.

SEAPlant.net Foundation (SPNF) began as an initiative of IFC – Advisory Services under the PENSA I program that ended its five year term in June, 2008. During jointly funded work involving the PENSA program and GTZ (Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.) it became clear that an integrated, ongoing and readily accessible body of information was necessary to facilitate and catalyze the development of seaweed farming as a component of integrated multi-trophic aquaculture (IMTA) in the BIMP-EAGA region in particular and in the Coral Triangle in general. GTZ therefore joined with SPNF to develop "*A Practical Guide to Quality Assurance, Governance Systems and Good Practices for Tropical Seaweed-to-Carrageenan Value Chains with focus on developing harmonization and transparency in the BIMP-EAGA region of ASEAN in the Coral Triangle*" (SEAPlant.net Monograph no. HB2D 1108 V1 GTZ). The practical guide is provided as a tool for negotiating the tangled web of rules, regulations, standards, tests and other requirements that increasingly make life complicated for industry stakeholders whether they be seaweed farmers, processors or end-users.

One of the objectives of the Practical Guide is to bring about the development of harmonized Laboratory Test Procedures (LTP) for seaweed farming within the region. The present document is a draft that we hope will ultimately lead to LTP.

This a "living document" that is being updated periodically. We heartily welcome suggestions and guidance from the users of the present monograph and the Practical Guide.

Iain C. Neish, October, 2008
Makassar, Sulawesi Selatan, Indonesia

GLOSSARY

Biopolymer - Compound of high molecular weight synthesised by living organisms

Carrageenan - Red algal galactan biopolymers produced by genera such as *Kappaphycus, Eucheuma, Betaphycus, Gigartina, Chondrus* and others.

DES - Dried Eucheuma Seaplants

ES - Eucheuma Seaplant (s)

ESVC - Eucheuma Seaplant (s) Value Chain (s)

LTP –Laboratory Test Procedure

MC – Moisture content

MW – Molecular weight

PES – Processed Eucheuma Seaweed = E407a

SS – Stainless Steel

Rheology - Textural characteristics of a gel or solution.

Seaplant - Any photosynthesising organism that lives in seawater

Seaweed - Common name applied to most marine macroalgae

SFDM - Salt free dry matter

BIBLIOGRAPHY FOR THIS SERIES OF MONOGRAPHS: Please download: "*A Reference List for Commercially Cultivated Tropical Red Seaweeds*." SEAPlant.net Monograph no. HB2G 1008 V3

Interpreting test results

Cottonii raw material tests...	
Water	Weight lost during desiccation in a drying oven is defined as being “moisture content”.
Sand	Material that does not dissolve after thorough soaking and rinsing is defined as “sand”.
Junk	Junk weed, tie-ties, debris and other foreign matter is simply removed and weighed.
Salt	Whatever disappears after thorough rinsing and drying is defined as being “salt”.

Salt-free dry matter includes...	
Carrageenan **fiber** **Lost organics**	Salt Free Dry Matter (SFDM) is also known as Clean Anhydrous Weed (CAW). It is left over after thorough rinsing and drying. It includes carrageenan, crude fiber (a.k.a. acid insoluble matter or AIM) and organic materials lost during cooking and washing.

PES tests...	
Water	Weight lost during desiccation in a drying oven is defined as being “moisture content”.
Sand	Should be absent in PES.
Junk	Should be absent in PES.
Salt	Should be present at low concentration in PES. A high salt means poor washing.

Salt-free dry matter includes...	
Carrageenan	Quantitative measurement of carrageenan requires extraction of the gum from PES (procedure not included here). Viscosity is a rough indicator of molecular weight and gel strength reflects the degree of modification, and carrageenan concentration.
fiber	fiber is what differentiates E407 from E407a.
Alkali	A high level means poor washing.

1. It must be recognised that component identities such as “sand”, “salt”, “moisture”, and others are correct only as defined by specific tests and are not necessarily true in a chemically definitive sense. These are component definitions that are adequate for practical commercial purposes.
2. PES is not pure carrageenan. Actual carrageenan may vary widely in both concentration and quantity. This, in turn, effects product performance in both rheological tests and applications.
3. Proper use and interpretation of rheological tests (e.g. gel-strength and viscosity) is used not only for quality assurance (QA) but also for quality control (QC). Process parameters can be adjusted to hit fixed rheological performance targets even if raw material is variable.
4. Low gel-strength accompanied by high viscosity indicates under-modification and/or “immature” raw material.
5. Low gel-strength accompanied by low viscosity indicates over-modification and/or degraded raw material.
6. Gel-strength can be adjusted somewhat by regulating the degree of washing - hence the salt content.
7. High fiber can indicate that gum is being locked into insoluble complexes by divalent cations (e.g. Ca++ and Mg++). This can be reduced by using an ion-exchange presoak and/or soft process water.
8. Note that during blending it is dangerous to reach on-specification gel-strength by mixing very strong with very weak batches. Such blends may pass gel-strength tests and still fail in applications. Strong and weak material respond differently to process conditions (e.g. the weak material depolymerising and “failing” in process).

INDEX & overview of Laboratory Test Procedures (LTP)

Overview of Cottonii Raw Material Laboratory Test Procedures

Component	LTP #	Purpose/Comments
Water	001	Weight lost during desiccation in a drying oven is defined as being “moisture content”.
Sand	007	Material that does not dissolve after thorough soaking and rinsing is defined as being “sand”.
Junk	Sort & weigh	Junk weed, tie-ties, debris and other foreign matter is simply removed and weighed.
Salt	006	Whatever disappears after thorough rinsing and drying is defined as being “salt”.
Salt Free Dry Matter (SFDM)		
Carrageenan	006 SFDM (CAW)	Salt Free Dry Matter (SFDM) is also known as Clean Anhydrous Weed (CAW). It is left over after thorough rinsing and drying. SFDM includes the carrageenan, crude fibre (a.k.a. acid insoluble matter or AIM) and organic materials that are removed from the SFDM during cooking and washing steps of the PES process.
Fibre		
Lost Organic Matter		

Overview of PES Laboratory Test Procedures

Water	002, 003	Weight lost during desiccation of PES in a drying oven is defined as being “moisture content”.
Sand	007	Should be absent in PES but may appear in trace quantities.
Junk	Sort & weigh	Should be absent in PES but may appear in trace quantities.
Salt	006	Should be present at low concentration in PES. A high level means poor washing.
Carrageenan	014 017	**Quantitative measurement of carrageenan** requires extraction of the gum from PES (procedure not included here). **LTP-014** measures viscosity and is a rough indicator of molecular weight (high viscosity indicates high MW). **LTP-017 is a** gel strength measurement that reflect the degree of modification, the carrageenan concentration and other qualities of the PES.
Fibre	009	This crude fibre is what differentiates PES form clarified carrageenan extract.
Residual Alkali from processing	010	Should be present as very low concentration in PES. A high level means poor washing.

Also includes LTP 028 for titrating alkalinity of modification solution.

Laboratory Test Procedures (LTP) 001 & 002

LTP # 001 Moisture in raw and washed-chopped seaweed

Materials
- Analytical pan balance (accurate to 0.01 gm.)
- Drying oven
- Aluminium trays

Procedure
1. Tare aluminium tray and record weight.
2. Fill tray with 25 gm. sample (raw cottonii and spinosum should be cut into 3-5 cm.); spread sample very thinly and evenly.
3. Dry at 60^0C (for raw spinosum) and 80^0C (for raw cottonii) for 18 hours. Don't load the oven with materials of widely variable moisture content.
4. Weigh tray and dry seaplant. Record weight.

Reagents
- none

Calculations:

Wt. of dry seaplant = (wt. of tray + dry seaplant) - (wt. of tray empty)

$$\% \text{ MC} = \frac{25 \text{ gm.} - \text{wt of dry seaplant}}{25 \text{ gm.}} \times 100$$

LTP # 002 Moisture in PES Chips

Materials
- Pan balance; accurate to 0.01 gm.
- Drying oven
- Aluminium drying pans

Procedure
1. Tare pan (to 0.01 gm.). Record as tare weight.
2. Fill pan with the raw material sample (25 gm.).
3. Weigh pan and sample. Record as original gross weight.
4. Dry at 80^0C for a minimum of 14 hours (80^o C for raw material and 60^o for chopped material).
5. Remove pan from oven and weigh. Record as final gross weight.

Reagents
- none

Calculations:
1. Subtract weight of pan (tare weight) from original gross weight. This equals original net weight.
2. Subtract final gross weight from original gross weight. This equals weight loss.
3. Divide weight -loss by original net weight and multiply by 100. This gives % moisture.

Note: Please save dried material for EDTA-wash analysis.

Laboratory Test Procedures (LTP) 003 & 006

LTP # 003 Moisture in PES Powder

Materials
- OHAUS Moisture balance; accurate to 0.01 gm. (10 gm. capacity)
- OHAUS Aluminium pan liner

Reagents
- None

Procedure
1. Turn the button switch to “on” position.
2. Place a pan liner on the pan and rotate the knob counter-clockwise until the zero mark lines up with the vernier zero.
3. Weigh 10 gm. sample on balance. Spread thinly and evenly on the pan.
4. Position heater unit about 3 cm. above the pan and set the heater control to 5.50.
5. Set the timer to 25 minutes. The heater will turn on and the test begins. As the sample is heated, it keeps on losing moisture.
6. The timer will sound a bell and turn off the heater unit when the 25 minutes has elapsed.

Calculations:

1. Read directly moisture loss from the optical scale.

Note: The reticle is graduated both in grams and in percent moisture. Since gram readings increase from 0 to 10 in one direction while the percentage readings decrease from 10 to 0 in the same direction, the vernier which permits the readings to 0.01 gm. and 0.10% is a split vernier. The upper half of the vernier is used when reading gram values, the lower half is used when reading percent values.

LTP # 006 Salt, Sand & salt-free dry matter (SFDM)… also called clean anhydrous weed (CAW) in eucheuma seaplants

Materials
- Analytical Pan Balance (accurate to 0.Ol gm.)
- Drying Oven Model 630
- Aluminum cafeteria trays
- Beaker, Pyrex, 4 litre capacity
- U.S. Standard Sieve No. 12 and No. 60
- Water Sprinkler
- Distilled or deionized water

Procedure
1. Weigh accurately 60 gm seaplant (DES spinosum and cottonii) pre-cut to about 4 cm. and place into a 4 litre beaker.
2. Add about 2 litres of water.
3. Soak the weed for 30 minutes with agitation every 5 minutes.
4. Drain off water using sieve No. 12 and spray rinse for about 2 minutes.
5. Tare cafeteria tray and record weight.
6. Dry weed on the cafeteria tray and spread very thinly and evenly (one particle thick).
7. Dry at 60^0C (for spinosum) and 80^0C (for cottonii) for 18 hours. Don’t load the oven with materials of widely different moisture content.
8. Weigh tray and washed anhydrous weed and record weight.

Reagents
- none

Calculations:

$$\%SFDM = \frac{(\text{wt. tray} + \text{wt. SFDM}) - \text{wt. tray empty}}{60\ \text{gm.}} \times 100$$

%S&S = 100 - (%SFDM + %MC)

Laboratory Test Procedures (LTP) 007 & 008

LTP # 007 Sand Determination

Materials
- Pan balance; accurate to 0.01 gm.
- Drying oven
- Aluminium cafeteria tray
- Bucket or beaker, 1 litre. capacity
- Filter cloth
- Distilled or De-ionised water
- Funnel

Procedure
1. Get a representative sample of approximately 100 gm. of sorted raw material.
2. Cut to about 1 inch and weigh accurately *25* gm.
3. Place into a 1 litre. bucket or beaker and add 500 ml. of water.
4. Soak DES for 30 minutes and stir occasionally.
5. Filter soak-water through pre-weighed filter cloth and wash weed twice times with 200 ml. water.
6. Wash the contents of the filter cloth thoroughly and dry to constant weight at 105^0C (about 3 hours).
7. Weigh filter-cloth and contents. Record as total weight.

Reagents
- None

Calculations:

Subtract weight of the filter cloth from the total weight and calculate proportion of "sand" (may include some dirt and plant material) in original sample.

C = [Total weight - wt. of filter cloth]

LTP # 008 Fibre

Materials
- Analytical balance, (0.01g)
- Steam bath
- Beaker, 250 ml. cap, Pyrex
- Watch glass to fit 250 ml. beaker
- Glass crucible with fritted disc; course, 30 ml. cap
- Drying oven, 105 C
- Desiccator
- Vacuum pump

Procedure
1. Weigh accurately l.50 gm. sample of whole, sorted material.
2. Transfer weighed sample into a 250 ml. beaker containing 150 ml. of distilled water and 15 ml. of 1.0% sulphuric acid.
3. Cover the beaker with a watch glass and digest the mixture on a steam bath for 4 hours, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation.
4. Filter through a tared glass crucible with fritted disc.
5. Wash the residue several times with hot distilled water, dry the crucible and its contents at 105^o C for 3 hours.
5. Cool in a desiccator and weigh.
6. Subtract the weight of the crucible empty from the weight of the crucible and residue to obtain the weight of the residue.

Reagents
- Sulphuric Acid, 1.0% solution

Calculations: Proportion of fibre (F)

F = wt. of residue / wt. of sample

Laboratory Test Procedures (LTP) 009

LTP # 009 EDTA - Yield

Materials
- Beaker, 250 ml. cap. Pyrex
- Magnetic stir bar
- Desiccator
- Analytical balance (0.Olg)
- Drying oven, 55 ^{0}C
- Vacuum oven
- Mikro-Mill with 60 mesh screen
- Pipette, 5 ml. cap
- Funnel
- Whatman filter paper #41
- Glass crucible with fritted disc, coarse

Reagents
- Alcohol / EDTA solution - 5g Tetra Sodium EDTA in 1000 ml. of 60% by weight IPA
- IPA (Isopropyl Alcoho]), 60% by weight
- IPA, 65% by weight
- IPA, 75% by weight
- IPA, 85% by weight
- HCl, concentrated
- NaOH, 1.0 N
- BDH Universal Indicator

Procedure
1. Powder about 10 gm. of anhydrous sample from the moisture analysis in a Mikro - Mill with 60 mesh screen.
2. Weigh accurately 0.50 gm of powder and transfer quantitatively to a 250-ml. beaker.
3. Add a magnetic stirrer bar and 100 ml. of EDTA/IPA solution.
4. Stir for 2 hours and then add 2.50 ml. of concentrated HCl and let stir for 10 minutes.
5. Remove stir bar and filter through pre-weighed #41 Whatman filter paper. Complete transfer with 65% IPA, being careful to wash all of the sample from the beaker. Wash the sample with successive additions of ca. 25 ml. of IPA (65%, 75%, 85%) to remove Na4EDTA.
6. Neutralise the sample with 1.0 N NaOH using BDH Universal indicator as a reference of pH.
7. Continue washing the sample with about 25 ml. each of 65%, 75%, 85% IPA in successive washes.
8. Transfer washed gum and filter paper to 55 0C oven and heat for 30 minutes.
9. Place in vacuum oven at 60% overnight. Allow cooling to room temperature in a desiccator.
10. Accurately weigh the dried gum on an analytical balance.
11. Subtract the weight of the filter paper from the weight of the gum and filter paper to get the weight of the gum.

Calculations:

$$E = R (I-M) / 0.50$$

Where: E = EDTA - wash yield

R = wt. of washed material recovered (gm.)

M = proportion of moisture in as - is sample

Note: For this analysis, use the dried sample from the moisture content analysis. Save the gum for LTP-010 analysis (alkali residue).

Laboratory Test Procedures (LTP) 010 & 014

LTP # 010 Alkali Residue

Materials
- Beaker, 100 ml. cap
- Analytical balance (0.01 gm.)
- Magnetic stirrer with magnets
- pH meter
- Burette, 50 ml. cap (acid and alkali)

Reagents
- 0.10 N HCl
- 0.10 N NaOH

Procedure
1. Measure from an acid burette 40 ml. of 0.10 N ,HCl and place into 100 ml. Beaker
2. Weigh 1.50 gm. of sample and suspend in 40 ml. of 0.10 N HCl.
3. Agitate with a magnetic stirrer for 4 hours.
4. Back-titrate the solution to pH 7.0 with 0.10 N NaOH and record volume of NaOH used.

% KOH (A) is calculated as follows:

$$A = \frac{(V_{HCl} \times N_{HCl} - V_{NaOH} \times N_{NaOH}) \times 0.056}{\text{grams of sample}}$$

NB: 0.056 is molecular weight of KOH/1000

Calculate the milliequivalents (meq.) of total alkali per gram of sample (AR) as follows:

$$AR = \frac{(V_{HCl} \times N_{HCl} - V_{NaOH} \times N_{NaOH})}{\text{grams of sample}}$$

LTP # 014 Viscosity and pH

Materials
- SS bucket, 1.2 litre capacity
- Propeller (3-blade)
- Boiling Water Bath
- Cold Water Bath
- Stirrer
- Thermometer (-20 º to +110^{0}C)
- Balance (0.01 gm. accuracy)
- Viscometer Brookfield LVT #1 Spindle,
- pH Meter
- Timer

Procedure
1. Disperse, with agitation, 7.5 gm. of sample in tared beaker with propeller, containing ~450 ml of distilled or deionized water. After thoroughly dispersed, bring to final weight of 500 g plus tare with distilled or deionized water.
2. Heat in water bath with continued agitation until a temperature of ~85ºC is reached. Adjust for loss by evaporation, cool to 76-77ºC and place in 75^{0}C temperature bath.
3. Preheat viscometer spindle and guard to ~75ºC. Dry and attach bob and guard to viscometer. Adjust height of spindle in solution. Start viscometer rotating and measure solution temperature.
4. Determine viscosity of solution at 75 ^{0}C with Brookfield viscometer using #1 spindle at 12 rpm for 30 seconds
5. Cool solution to 30^{0}C and measure pH.
6. When measuring the viscosity of high-viscosity gums, use a #2 spindle and read the results on the 0-100 scale. Otherwise, proceed as above.


Brookfield LVT

Reagents
- none

Calculations:
Calculate viscosity in terms of millipascal seconds (mps).

LTP # 017 PES Water Gel

Materials
- Stevens LFRA Gel tester
- Small head plunger
- 1 litre. Pyrex reagent bottle
- 10 ml pipette (0.1 ml gradation)
- Crystallising dish, 70 x 50 mm
- 1000 gm. cap.spring balance
- l.2 litre SS bucket
- Propeller (3-blade)
- Stirrer
- Boiling H_20 bath
- Balance (0.01 gm.)
- Small flat-bladed spatula
- Weigh boats
- Cooling water bath

Stevens LFRA

Reagents
- Calcium Chloride Dihydrate ($CaCl_2.2H_20$)
- Potassium Chloride (KCl)

Procedure
1. Prepare salt solution by dissolving 100 gm. KCl and 100 gm. $CaCl_2.H_20$ in distilled H_20 and bringing to a volume of 1 litre with distilled H_20. Store in sealed 1 litre Pyrex bottle.
2. Tare bucket and propeller.
3. Add 10.0 ml salt solution to bucket.
4. Bring volume to approximately 485 ml with cold H_20.
5. Weigh 10.0 gm. sample and add to bucket using agitation.
6. Continue agitation for approximately 2 minutes.
7. Place bucket in boiling H_20 bath.
8. Heat, using agitation for 15 minutes.
9. After 15 mm., remove bucket from bath, wiping excess water from outside of bucket.
10. Bring solution to 500 gm. net using distilled H_20.
11. Mix thoroughly.
12. Pour solution to top of three crystallising dishes and set immediately in trays with cold water for 15 minutes. Transfer dishes into the cooling water bath and set gels for 45 minutes.
13. As temperature of gel reaches 25°C, invert gel and replace into dish.
14. Make one determination of break -force on each gel using LFRA gel tester, small head plunger and appropriate balance.

Calculations:

Report gel strength in grams break-force by averaging the three results. Also report the individual readings.

Laboratory Test Procedures (LTP) 028

LTP # 028 Titration of KOH Solution

Materials
- pH meter
- Burette, acid, 50 ml cap
- Beaker, Pyrex, 500 ml cap
- Beaker, Pyrex, 400 ml cap
- Stirring rod
- Volumetric flask, 100 ml
- Volumetric flask, 200 ml
- Volumetric flask, 2000 ml.

Procedure
1. Collect about 300 ml K0H liquor in a 500 ml beaker.
2. Mix well and measure 100 ml into a 100 ml volumetric flask.
3. Transfer the liquor to a 2 litre volumetric flask and dilute to volume with deionized water. Mix well.
4. Take 200 ml aliquot portion and transfer to 400 ml beaker.
5. Add 3 drops phenolphthalein indicator and titrate with 1.00 N Sulphuric acid to pH 8.20. Record acid volume consumed.
6. Add 3 drops methyl orange indicator and continue titration with 1.00, N Sulphuric acid to pH 4.30. Record final volume of acid.

Reagents
- 1.00 N Sulphuric Acid
- 1.00 N Sodium Hydroxide (to standardise 1.00 N Sulphuric Acid)
- Potassium Acid Phthalate (to standardise 1.00 N Sodium Hydroxide)
- Buffer Solution, pH = 7.0
- Phenolphthalein indicator
- Methyl orange indicator
- Deionized water

Calculations:

20.0 ml of 1.00 N sulphuric acid titrates a sample containing KOH + K_2C0_3 to the phenolphthalein end point. An additional 5.0 ml of the acid is required to continue the titration to the methyl orange end point. Compute normality of KOH.

$$\mathbf{N_{KOH} = (20.0 \times 2.0) \times (1.00) - (25.0 \times 1.00)/10^*}$$

(*aliquot portion of KOH liquor 100 x 200/2000)