Seaweed Projects funded by PCAMRD

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This paper reports the different seaweed projects funded by the Philippine Council for Aquatic and Marine Research and Development (PCAMRD). The research areas are focused on inventory and assessment, and processing and utilization of the economically important seaweeds of the Philippines (*Eucheuma, Gelidiella, Gracilaria, Gracilariopsis, Kappaphycus,* and *Sargassum*) see following table.

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Title/ Project leader	Objectives	Accomplishments
INVENTORY AND ASSESSMENT		
1. Inventory, assessment and utilization of agarophytes and alginophytes in the waters of northern Mindanao - **Wilfredo Uy**, MSU-Naawan	General Objectives • To determine the sustainable yield levels of *Sargassum* spp. & *Gracilaria* spp. in Northern Mindanao • To determine the effect of seasonality & biomass levels on the quality of agar and algin • To provide immediate & preventive measures to sustain production & initiate assessment of these measures	
1a Assessment of *Sargassum* spp. & *Gracilaria* spp. in the waters of Northern Mindanao- **W. Uy**	Specific Objectives: • To identify species of *Sargassum* & *Gracilaria* found along the coastal waters of Northern Mindanao (including its macroflora and faunal associates) • To determine the seasonality and biomass levels of *Sargassum* & *Gracilaria* spp. in the area • To determine physico-chemical parameters in the area and correlate these with seasonality, species richness, zonation and algin/agar quality	• From the 6 stations established, 116 species of macrobenthic algae were identified. There were 49 red, 30 green, 25 brown and 4 bluegreen algae. *Sargassum* has 8 species while *Gracilaria* has 9 species with an unknown species from Camiguin island. • The project facilitated the approval of a local municipal ordinance prohibiting the harvest of *Sargassum* along the coast of Naawan. This was done after a series of public hearings participated in by researchers, local officials and seaweed collectors

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l.b Assessment of agar and alginate quality of *Gracilaria* & *Sargassum* spp. in Northern Mindanao area - **Grace Prado**	• To assess the physical quality of agar and alginate from the different *Gracilaria* and *Sargassum* species, respectively • To determine seasonality of agar and alginate quality	• In the stations surveyed, *Sargassum* has the widest bed except in Lala, Lanao del Norte where there was none because of the muddy substratum. *Gracilaria* was found to be abundant in this area. Results showed that *Sargassum* spp. are generally abundant in Maigo station with 40% algal cover during January and April. • For *Gracilaria,* Initao station has the most number of species and it is generally present throughout the year. • The most dominant macrobenthic algae was, *Sargassum crassifolium,* followed by S. *ilicifolium, Gelidiella acerosa* & *G. arcuata.* Other species identified include *Padina* spp., *Turbinaria conoides, Laurencia papillosa, G. coronopifolia, Ulva reticulata* & *Cladophora* sp. • Data gathered indicates that *Sargassum* was generally vegetative. • Samples of agar from *Gracilaria* & alginate from *Sargassum* were analyzed at the UP Marine Science Institute.
l.c Management strategies for the conservation & utilization of agarophytes and alginophytes in Northern Mindanao - **G. Prado**	• To develop community-based management of seaweeds in Northern Mindanao with a focus in *Sargassum* and *Gracilaria*	
2. Utilization of agarophytes in the waters off Ilocos Norte -**A. Ragasa** 2.a Inventory/assessment and utilization of agarophytes in Ilocos Norte	• To determine the seasonal variation of yield and quality of agar from the various species of agarophytes • To evaluate and assess the physico-chemical qualities of the agar • To correlate seasonal variation with agar quality • To develop products and test-purify agar extracts as culture media for microorganisms	There were five dominant agarophytes collected namely *Gracilaria coronopifolia, G. eucheumoides, G. salicornia, Laurencia flexilis* and *Gelidiella acerosa.* The highest agar yield was registered from *G. coronopifolia,* in the month of April. Agar yield could be attributed to the stage of growth and development of the seaweeds. *G. acerosa* had the highest gel strength while *G. coronopifolia* the lowest. Viscosity of agar showed *L. flexilis* to be the highest with *G. coronopifolia* as the lowest. The sulfate content analysis showed *L flexilis* and *G. coronopifolia* to have high values with *G. acerosa* having the least value. *G. salicornia, G. acerosa* and *G. eucheumoides* have high values in 3,6 anhydrogalactose while minimal values was registered by *G. coronopifolia.* High ash content was shown by *L. flexilis* while *G.*

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		acerosa has the least. The gelling temperature of L *flexilis* was highest and *G. coronopifolia* the lowest. As to the melting temperature, *G. acerosa* had the highest while *G. eucheumoides* the lowest. Significant correlation between yield and physico-chemical properties of extracted agar were observed among the agar extracts. Bio-physico-chemical factors were significantly related to yield. All agar extracts were acceptable but in the product formulation, *Gelidiella acerosa* received the highest acceptable rating. Microbial examination of the agar gave minimal microbial counts. Purified agar extracts from the agarophytes could be used as culture media for selected microorganisms.
3. Inventory, assessment and utilization of agarophytes and alginophytes in the waters of Cebu and Bohol - **F. Sotto**		
4. Inventory, assessment and utilization of agarophytes and alginophytes in the waters of Negros Occidental and Oriental **H. Calumpong**		
5. Assessment, management and utilization of agarophytes and alginophytes in selected coastal areas of Zamboanga and Basilan waters - **P. Domingo, F. Saupi, M. Ontolan and F. Piedad**		
6. Assessment, management and utilization of agarophytes and alginophytes in		

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selected areas in Cagayan waters - **R. Domingo**		
7. Training Program and Coordination on Taxonomy of *Gracilaria* and Processing of agar - **N. Montaño**		
8. Inventory and assessment of *Gracilaria* and *Sargassum* in selected areas of the Philippines **G. Trono, Jr. -** UPMSI **R. Sariego -** SPCP **J. Manzano** - BUTC **E. Mamaril -** DMMMSU	• To inventory and assess the natural stocks of *Gracilaria* and *Sargassum* in selected areas in the Philippines • To determine the seasonality in standing crop and reproductive phenology of the stocks • To formulate and implement managed harvest schemes for the utilization and conservation of the stocks	Inventory and assessment of *Sargassum* and *Gracilaria* were conducted at selected areas in the country using the method of Saito and Atobe. Sargassum stocks reach their peak growth, standing crop and fertility during the cold months - November to December and die-off the following months of January-February. Regeneration and growth resumed afterwards. Large and well-developed *Gracilaria* stocks appeared to be limited in their distribution in highly fertilized waters in coves, lagoons, near mouth of rivers, and landward portions of reef adjacent to mangrove communities during the summer months of April, May and June and disappeared during the rainy month of July to December.
9. Development of *Gracilaria* cultivars from seaweed farms in open water - **G. Trono, Jr.**	• To select and develop fast growing, high yield and good agar quality *Gracilaria* species/cultivars for the seaweed farms in open waters Specific Objectives: • To compare the growth of the different species in controlled conditions and to use the culture materials for agar extraction and characterization. • To compare the agar quality of the cultured materials to field collected materials of the same species. • Using the results in objectives 1 and 2, to select the species or strain with high agar yield and quality for mass production as cultivars for commercial farming.	Three local agarophyte species (*Gracilaria firma, Gracilaria* sp. and *Gracilariopsis bailinae*) were grown under controlled outdoor flow-through culture conditions. Growth rates and agar characteristics of the three species were determined. *G. firma* showed superior growth and agar quality among the three species. It exhibited the highest growth rate, highest agar gel strength and was observed to be highly resistant to epiphytes. Growth experiments under various light fluence and ammonium combinations showed that the highest light fluence level and moderate ammonium concentration resulted to highest growth rates for all species. The single and interactive effects of light fluence and ammonium enrichment on growth and agar characteristics of the three species were highly significant. Field culture of the three species is feasible though seasonal. *Gracilariopsis bailinae* is suitable for pond culture while *G. firma* and *Gracilaria* sp. are best suited in open water culture.

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	• To develop hatchery techniques in the mass production of the selected species. • To conduct field culture trials using the hatchery produced cultivars and finally extend this technology to farmer cooperators.	
10. Development of germling production and culture technology of *Sargassum* - **G. Trono, Jr.**	• To develop the technology for the hatchery production and culture of *Sargassum* germlings • To conduct field studies on the rehabilitation of over-exploited/ecologically stressed *Sargassum* beds and development of *Sargassum* bed. Specific Objectives: • To establish the individual and combined effects of photon flux density, culture media and salinity on the growth and survival of *Sargassum crassifolium* germlings under laboratory conditions • To determine the optimum laboratory conditions for the growth and survival of *S. crassifolium* germlings • To compare the growth and survival of *S. crassifolium* juveniles cultured in the laboratory and transferred to hatchery and field conditions • To determine the individual and combined effects of photon flux density, culture media and salinity on the growth and survival of juveniles in the hatchery • To know the recruitment capacity of *Sargassum* germlings on artificial substrates and recommend the	The effects of photon flux density, culture media and salinity on the growth and survival of *Sargassum crassifolium* germlings shed on artificial substrates showed that the main and interactive effects of salinity and photon flux density significantly affected the growth of the germlings during the entire culture period. Growth was enhanced with the addition of media during the later part of development. Survival rate was shown to be influenced mainly by photon flux density with higher rate at 100 than at 200 $uEm^{-2} s^{-1}$. In the hatchery, where the germlings were transferred for further growth, the most favorable culture condition was filtered seawater with ES enrichment at 35 ppt salinity and 300 $uEm^{-2} s^{-1}$ photon flux density. The interactive effect of salinity and photon flux density appeared to be more deleterious than their individual effect. High mortality rate observed on the germlings deployed in the field was due to grazing. The germlings had to be grown to plantlets further in the hatchery before being deployed in the field. Results of *in situ* recruitment studies on concrete cement blocks showed that more germling were recruited on the blocks deployed during the colder months of October to January coinciding with the fertility peak of *Sargassum* populations in the area. Blocks during low fertility months were overgrown by other algal species preventing the settlement of *Sargassum* germlings. The best time recommended for substrate deployment would be September to December. The recruitment capacity of the *Sargassum* populations appeared to vary with time. Environmental parameters monitored did not seem to have a significant effect on the recruitment rate. Grazing appeared to be an important factor affecting recruitment.

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	period for substrate deployment. • To determine the feasibility of establishing *Sargassum* beds using spore bag technique	
SEAWEED PROCESSING AND UTILIZATION		
1. Development of fertilizers/ growth hormones from Philippine seaweeds - **N. Montaño & A. Corpuz**	• To develop seaweed sources of low-cost fertilizers and/or growth hormones Specific Objectives • To develop fertilizers/growth hormones from seaweeds and seaweed waste through different extraction procedures • To assay for the growth hormonal substances present in these fertilizers • To analyze the micronutrient contents of the seaweed and the seaweed extracts • To conduct pot and field experiments to determine the effectivity of the fertilizers. • To determine the effective concentrations for seaweed fertilizer applications • To conduct laboratory experiments to ascertain the effectivity of seaweed extracts in delaying the ripening of fruits and the wilting of cut flowers.	*Sargassum* extract was tested as fertilizer for chrysanthemum, pechay, strawberries, carrots, potatoes, tomatoes, lettuce and as Postharvest treatment for tomato and chrysanthemum. Results showed that there was no significant effect on the growth performances of the above test crops when it was used as fertilizer. On the other hand, application of the seaweed extract significantly affected the final height of chrysanthemum. Its effect on the number of days from flower bud formation to anthesis and flower diameter was comparable to the effect of the use of commercial with chicken manure fertilizers. The combination of seaweed extract and inorganic fertilizer gave a significant effect on the growth and yield of potato. Hot processed seaweed extract at 0.5% was seen to be most effective when used as a supplemental fertilizer for tomato. Postharvest studies of salad tomato showed that ripening was delayed in fruits treated with 1.0% seaweed extract while increased seaweed extract concentrations of 1.5% and 2.0% hasten ripening of tomato fruits. Holding cut chrysanthemum flowers in 1.0% and 0.5% seaweed extracts resulted in delayed flower deterioration as exhibited by better overall visual quality, better foliage and prolonged vaselife.
2. Search for new sources of carrageenan from Philippine seaweeds and the development of applications for unmodified carrageenan - **N. Montaño**	• To search for new sources of *lambda* carrageenan from Philippine seaweeds • To search for new sources of unmodified carrageenan *(kappa, iota, mu, nu* and *lambda)* from Philippine seaweeds • To determine the physico-chemical properties of *lambda* and unmodified	Three seaweeds, namely *Hypnea* sp., *Bostrychia* sp., and *Eucheuma gelatinae* were identified as potential sources of carrageenan. These sources were identified on the basis of yield, and conformity with the regulations as stated in the Food Chemicals Codex 3rd ed. The results showed percentage yield and infra-red spectroscopy information. On the other seaweeds, data pertaining to the sulfate content were also collected and for those extracted polysaccharides that were able to form a gel, the melting point and gelling point of the gel in the presence of various ions were determined to establish the polyssacharide's ionic

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		reactivity. Although *Gelidium* sp. and *Gracilariopsis* sp. are identified as agarophytes, the experiments revealed that their polysaccharides react with methylene blue, and contain a high sulfate content from which one might infer that they are carrageenans. However, these data also point to the possibility that the polysaccharides may be similar to fumoran, a sulfated agarose obtained from the seaweed *Gloiopeltis furcata.*
3. Nutritional evaluation of Philippine semi-refined carrageenan - **L. Panlasigui**	• To evaluate the nutritional benefits of carrageenan-incorporated foods. Specific Objectives: • To formulate and evaluate the nutritional composition of food products with carrageenan such as pan de sal, fishball, longganiza, hamburger patty, gelatin and gumdrop (at varying levels of carrageenan) • To investigate the effects of carrageenan incorporation into food products on blood glucose level	Nutritional evaluation showed that carrageenan incorporation increases the dietary fiber content of the tested food items from 1.60% to 58.40%. Pan de sal and fishball with varying amounts of dietary fiber ranging from 10.95 to 32.57 g were given to normal and non-insulin dependent diabetic subjects. Control and experimental food samples were calculated to contain 50 g of total available carbohydrate. Results show that pan de sal formulation 1 did not significantly reduce the post prandial glucose rise in normal and diabetic subjects. It appears that for fiber to have an effect on blood glucose response, more than 12 g dietary fiber has to be present in 50 g available carbohydrate. The fried fishball study results showed that carrageenan incorporation significantly reduced blood glucose levels. Soluble fiber has been shown to increase viscosity of luminal content, and therefore delay the digestion and absorption of some nutrients such as carbohydrates. This accounts for the reduced post-prandial glucose levels. For blood glucose to be reduced significantly, the ingredients should be properly proportioned and manipulated aside from containing more than 12 g dietary fiber per 50 g available carbohydrate portion of the food.
4. Development of new carrageenan products from selected Philippine red seaweeds - **N. Montaño**	• To screen different sources of *mu, nu, lambda* and other carrageenans • To determine the physico-chemical properties of the different carrageenan such as gel strength, viscosity, gelling and melting temperatures, sulfate and 3,6-anhydrogalactose contents and others. • To determine whether these	The different samples of *Eucheuma spinosum* and *Kappaphycus alvarezii* collected from Bolinao, Pangasinan and Cebu were subjected to three different methods of extraction, two of which were modified. The first modification is the alkaline treatment wherein most of the carrageenophytes yielded products with higher viscosity, gel strength, gelling and melting temperatures and 3,6-anhydrogalactose content and lower sulfate content. The other treatment is borohydride, which yielded carrageenans with lower viscosity and gel strength as well as the gelling and melting temperatures. It also lowered the 3,6

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	carrageenans could be used for food and technical products such as beer, milk and others.	anhydrogalactose content of carrageenan extracted from *E. spinosum*. The carrageenans extracted were used in the formulation of fruit-flavored candy gels and pastilles. An air freshener gel was also formulated using a certain carrageenophyte. These said products have their patents pending.
5. Mass production of mannitol from *Sargassum* seaweeds - **N. Montaño**	• To develop an efficient method of mass extraction and purification of mannitol and to further characterize the extracted mannitol by chromatography, IR and NMR and molecular weight determination.	*Sargassum* collected from the sampling sites were screened for % mannitol content using periodic method. The most successful method of extracting mannitol from the seaweed involves the use of an acid solution with subsequent purification by crystallization or ethyl alcohol.
6. Pigments from Philippine marine sources - **B. Glorioso**	• To develop the technology for extracting pigments from various marine sources specifically the seaweeds.	Organic solvent and water soluble pigments from the following seaweeds have been extracted: *Halymenia durvillaei, Acanthophora spicifera, "Laurencia tronoi", Sargassum* sp. and a genetically improved seaweed from Nova Pacific Research Institute called *Gracilaria* NBr-10. Different procedures were tried in pigment extraction. Hot and cold and polar and non-polar solvents were used to determine the different solubilities of the pigments. Of the seaweeds screened for pigments, the red seaweed, *H. durvillaei* was given more attention since it yielded a bright red water soluble pigment which showed two separate peaks by high performance liquid chromatography (HPLC) The red pigment could be well utilized as food colorant but would find more valuable application as fluorescent tags with numerous applications in flow cytometry, fluorescent activated cell sorting, histochemistry, in immunoassays and also in the detection of relative oxygen species. The brown seaweed *Sargassum* sp. yielded two types of pigments constituting the polar and non-polar fractions. The polar fraction contained light brown to orange water-soluble pigments while the non-polar fraction yielded pigments believed to be composed of fucoxanthins or carotenoids. The carotenoids would be quite valuable as antioxidants, food supplement and as food.
7. Development of Philippine seaweed polysaccharide for food and industrial	• To develop the bench scale technology for preparing phycocolloid derivatives, phycocolloid based/stabilized	Products incorporating algal polysaccharides were developed. Interactions of carrageenan with some food and industrial substances such as glycerol, albumen, gelatin and antibiotics were also

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application - **N. Montaño**	food and pharmaceutical formulations • To determine the shelf-life of the formulated products	investigated. The products formulated are as follows: Halimuyak Gel Soap, Halimuyak Liquid Soap, Ginhaua Ointment, Suppository Base, Low Calorie Jelly, Egg Coatings, Decorating Gel, Instant Gum Paste and Low Calorie Maple Syrup
8. Screening of high quality agar from Philippine red seaweeds and development of appropriate processing techniques for agar - **N. Montaño**	• To screen different *Gracilaria* species and red seaweeds for good quality agar • To develop appropriate processing techniques for improving agar quality especially its gel strength • To test and classify the agars whether they are food, sugar reactive or micro-biological grade • To characterize and elucidate the structure of the extracted agar • To undertake shelf-life studies on the extracted agars	*Gracilaria* species as well as other equally important agarophytes which were collected from different parts of the country were screened for good quality agar. Native agar was extracted by boiling the algal samples in water for one hour. However, pressure cooking (15 psi at 120°C for an hour) was employed in the extraction of native agar from seaweeds that possess hard thalli. A combination of irradiation at kGy and pressure-cooking is a good extraction procedure for *Gelidiella acerosa* while G. *eucheumoides* exhibited better agar quality when pre-treated with 10% NaOH at 90° C for 2 hours. *G. eucheumoides* needs to be processed immediately after collection if high gel strength is desired, however, it could still be stored for a max of 6 months where Theological properties are still optimum. The seasonal evaluation on the quality of agar showed that *G. acerosa* registered high agar yield and gel strength during the rainy months between July and September while better quality agar was extracted from *G. eucheumoides* in the month of July. *G. edulis* registered maximum yield, and gel strength and other rheological properties during the month of May. Chemical analysis and spectrophotometric data revealed that agar extracted from the different seaweeds is composed mainly of 3-linked b-galactose and 4-linked 3,6-anhydro-a-L-galactose as the dissaccharide repeating unit. However, substituents like methyl ethers, sulfate esters and pyruvate ketals were present at varying degrees which explain the differences in the gelling properties. Alkali-treated agars from *Gracilaria eucheumoides, G. arcuata, G. tenuistipitata, G. edulis, G. salicornia, G. firma, Gelidiella acerosa, Gracilariopsis heteroclada* and *Laurencia flexilis* exhibited a maximum gel strength of 430, 278, 606, 225, 220, 820, 947 and 200 g cm^{-2}, respectively. Of the agarophytes studied, *G. eucheumoides, G. salicornia, G. firma* and *Laurencia flexilis* are potential sources of sucrose-reactive agars since their agar gels were more than two times stronger when added with 50% sucrose which possessed high water holding capacity.

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9. Interaction of paralytic shellfish poisons (PSP) with algal polysaccharides - N. **Montaño**	• To assess the interaction of algal polysaccharides with the red tide toxins and to develop their use as PSP antidotes Specific Objectives • To determine conditions for toxin-algal polysaccharide interactions, i.e., pH, temperature, concentration, etc. • To compare the interactions of red tide toxin with algal polysaccharides, ion exchange resins, and C18 resins • To conduct *in vivo* and *in vitro* assays of the toxin • To develop other tests for the toxin in conjunction with proposed IAEA project on red tide	The poisons used in the study were both crude extracts. PSP was extracted from *Pyrodinium bahamense* var. *compressum* and puffer fish poison was extracted from puffer fishes. When tested in mice, both crude extracts were toxic and hence were presumed to be positive of the toxins. PSP extracts were confirmed of the presence of saxitoxin (STX) but the puffer fish poison was not confirmed of the presence of tetrodotoxin (TTX) In the assessment of the in vitro interaction of PSP with algal polysaccharide, it was found that the gels of alginates and the carrageenans can reduce toxicity of the poison. The sol form of these polysaccharides does not have toxicity - reducing properties but seem to show toxicity potentiation properties instead. However, the gel form of these polysaccharides has very good poison-sequestering properties. When k-carrageenan was used as a model of polysaccharide gels, the partial characterization showed that the interaction was surface area-dependent, polysaccharide concentration-dependent and interaction-time dependent. It was also shown that the interaction was irreversible in isocratic conditions and was negatively affected by the presence of salts and proteins. The *in vivo* experiments on PSP showed that there was a delay in the onset of symptoms of paralytic shellfish poisoning. The *in vitro* assessment of the interaction of pufferfish poison with the carrageenans showed that the three carrageenan types can sequester the poison especially in the gel form. When k-carrageenan was used as a model, it showed that puffer fish poison-carrageenan gel interaction is concentration and time-dependent. Unlike with PSP, toxicity of puffer fish poison can also be reduced using the sol form of carrageenan.
10. Studies on the properties of an algal polysaccharides and its interaction with other gums: carrageenan - **N. Montaño**	• To search for galactomannans or mannans from Philippine indigenous sources which are synergistic with carrageenan • To determine the physico-chemical properties of mixtures of different carrageenans and native gums	

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	like ipil- ipil seed gum, galactomannans and mannans, industrial and indigenous gums • To serve as a clearing laboratory for carrageenan that is to be used for chemical derivatization to produce related to material science	
11. Management of natural stocks and the development of farming and processing technologies for *Gracilaria* for the socio-economic upliftment of the coastal communities in Northern Mindanao - **B. Que**	• To develop appropriate farming and processing technologies for *Gracilaria* and disseminate this technology to fisherfolks for the development of alternative employment opportunities and improvement of their socio-economic conditions. Specific Objectives • To develop appropriate farming techniques for *Gracilaria,* including evaluation of agar quality, test farming and determine the viability of culturing *Gracilaria* singly or together with prawns, crabs, shellfish or finfish in ponds, cages and/or in the open sea • To organize fisherfolks for farming by putting up demonstration farms as seedling banks, develop manuals and conduct seminars on farming and processing not only to fisherfolks but also to NGOs, GOs and private parties who can help in the development of the industry. Free consultancies/ technical assistance will also be provided to farmers.	The five coastal areas surveyed include Guinsiliban, Benoni, Mantigue, El Salvador and Balingasag. The different varieties of *Gracilaria* propagated came from Ubay, Bohol; Laguindingan, Misamis Oriental and from Kalawisan, Cebu. A seedling bank in Laguindingan was installed and a farming manual was developed. Three core groups were organized for *Gracilaria* farming and all of them are ready to proceed expansion for their *Gracilaria* farms. Three manuals were published: *Gracilaria* Farming, Agar Processing, and Food Applications of Agar. A product called AC10 was developed. Several food applications with agar as stabilizer were developed - Chocolate Cream Special, Instant Leche Flan, Peanut Gulaman Candy, Fruit Jello, Gulaman Peaches Dessert, Mango Ice Cream, Cream Fruited Squares, Mango Float, Nata Choco Jello, Langka Float, Corn Gulaman Special, Pineapple Gulaman Special, Cream Gulaman Special, Gulaman Float